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BEARING ON HEREDITY AND VARIATION

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A FRAGMENTED CHROMOSOME IN TRITICUM MONOCOCCUM AND ITS USE IN STUDIES OF INHERITANCE[1]

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Received February 26, 1947

VARIOUS sorts of chromosomal aberrations have been studied in order to determine their effects on the inheritance of genes and to determine the locations of genes in the chromosomes. These aberrations include duplications of whole chromosomes and segments of chromosomes (both free and attached), deficiencies of whole as well as parts of chromosomes, inversions, and interchanges.

The present paper expands on the abstract published by SMITH (1946) on the study of two fragments of a chromosome in *Triticum monococcum* L. (n = 7) that apparently arose as the result of breakage at the centromere. The fragments were first discovered in an individual offspring of a spike that had developed on a plant grown from an X-rayed dormant seed. Other plants in the progeny of the spike were normal.

Cytological observations were made on acetocarmine smears (SMITH 1947a).

CYTOLOGICAL OBSERVATIONS

In the original plant in which the two fragments were first observed and in plants in subsequent progenies that had a similar chromosomal constitution, chromosome size and synaptic behavior at diakinesis and metaphase *I* (A to J, figure 1) suggested the nature and probable origin of the two fragment chromosomes. In many microsporocytes six normal bivalent chromosomes were present plus three other chromosomes that frequently formed a "trivalent" complex. One of these three chromosomes was normal and had a submedian centromere. The other two were fragment chromosomes. The shorter fragment almost invariably associated with one arm of the normal chromosome (A, B, C, figure 1) and appeared to be of the same length as this arm. The other longer fragment chromosome frequently associated with the other arm of the normal chromosome. When in some sporocytes the longer fragment chromosome was not associated with the normal chromosome, but was present as a univalent, its two arms usually were associated with one another to form a ring-shaped synaptic configuration (B, C, figure 1). Again, although both fragment chromosomes associated with the normal chromosome in the manner

[1] This work was carried out in cooperation with the MISSOURI AGRICULTURAL EXPERIMENT STATION. Contribution from the FIELD CROPS DEPARTMENT, MISSOURI AGRICULTURAL EXPERIMENT STATION, Journal Series Paper No. 1039. Most of the data presented in this paper were obtained while the writer was with the U. S. DEPARTMENT OF AGRICULTURE, stationed at the UNIVERSITY OF MISSOURI.

indicated, they did not associate with one another. The combined evidence led to the conclusion that the shorter of the two fragment chromosomes was a telocentric chromosome composed of one arm of one of the normal chromosomes of the complement, and the longer fragment was an isochromosome composed of two identical arms that were homologous to the other arm of this same normal chromosome.

Because the two compensating fragments of one chromosome of the normal complement arose in the progeny of a plant that had been irradiated in the embryo stage, it was assumed that the following occurred: The irradiation resulted in the breakage of a chromosome within the centromere, and each arm retained a portion of the centromere. Both segments of the divided centromere were capable of functioning in the spindle figure (McCLINTOCK 1932, 1938). Consequently, two telocentric chromosomes took the place of the original normal chromosome. Subsequent to breakage, an isochromosome was formed from one of the telocentric chromosomes. The second telocentric

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FIGURE 1.—A to G Configurations in microsporocytes of plants with 13 normal chromosomes plus two fragments.

A. Diakinesis showing the two fragments pairing with a single normal chromosome (11 o'clock).
B. Diakinesis showing one fragment pairing with the normal chromosome and the other fragment nearby doubled back and apparently pairing with itself (11–12 o'clock).
C. Similar to B but showing only the normal chromosome and the two fragments.
D. First metaphase showing six closed bivalents and a zigzag trivalent made up of the telocentric, normal, and isochromosomes.
E. First metaphase showing six bivalents and a rod-shaped trivalent. Two of the bivalents are open and they, as well as one end of the trivalent, show that the centromeres are subterminal. The normal and telocentric chromosomes may go to the same pole from such a configuration.
F. First meiotic metaphase showing three closed and three open bivalents, a heteromorphic bivalent, and the isochromosome which in this case is open. Note again the appearance of subterminal centromeres on seven of the eight ends of the four open pairs.
G. First meiotic metaphase showing particularly a ring univalent (the isochromosome, 12 o'clock) in comparison with two accidentally unpaired univalents.

H to J Configurations in microsporocytes of plants with 14 normal chromosomes plus the telocentric chromosome.

H. First meiotic metaphase showing six closed bivalents plus the short, rod-shaped univalent.
I. First meiotic metaphase showing the telocentric chromosome pairing with one of the normal chromosomes and apparently leaving the other as a univalent nearby.
J. First meiotic metaphase showing six bivalents and a trivalent that could not be distinguished from a trivalent in plants with both fragments (for example D). All ×700

FIGURE 2

A. First meiotic anaphase in a plant with both fragments. At 11 o'clock is the telocentric chromosome. Below are the daughter chromatids of another chromosome (probably the isochromosome) that is dividing equationally.
B. First meiotic anaphase in a plant with 14 normal chromosomes plus the telocentric chromosome. The telocentric chromosome (12 o'clock) did not divide equationally in this cell. ×700

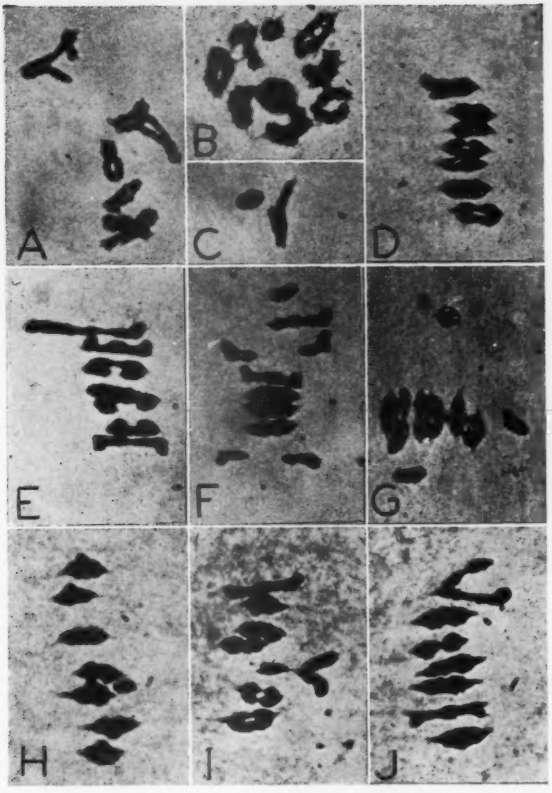

FIGURE 1

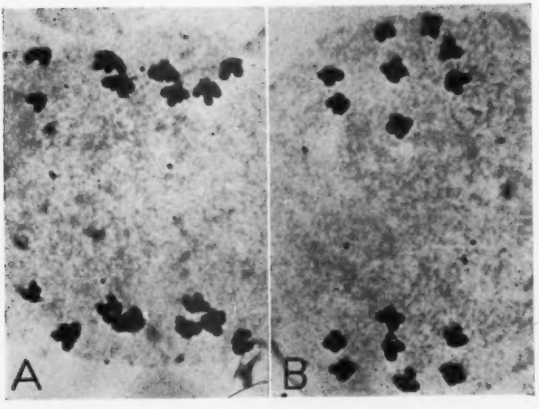

FIGURE 2

chromosome remained telocentric during the course of this study; no isochromosome derived from it having been observed.

When a "trivalent" complex, composed of a normal and the two fragment chromosomes, was present at first meiotic metaphase, the orientation of the members of the complex with respect to the spindle axis was not random. The most frequent orientation resulted in the disjunction of the normal chromosome from the two fragment chromosomes. RHOADES (1940) observed a similar nonrandom disjunction of the members of a "trivalent" complex that was composed of two normal chromosomes and a telocentric chromosome.

Data on microsporogenesis in plants with one or both fragment chromosomes are presented in table 1.

Plants with both fragment chromosomes gave rise to the following types of

TABLE 1

*Observations on meiosis in plants with one or both fragments.**

PLANTS	FIRST METAPHASE		FIRST ANAPHASE		FIRST TELOPHASE			SECOND TELOPHASE			
					CELLS WITH INDICATED NUMBER OF MICRONUCLEI			QUARTETS WITH INDICATED NUMBER OF MICRONUCLEI			
	CELLS WITH TRIVALENTS	CELLS WITH UNIVALENTS	CELLS WITH CHROMATIDS SEPARATING	CELLS WITH CHROMATIDS NOT SEPARATING	0	1	2	0	1	2	3 or more
Both fragments	179	192	79	154	609	17	2	226	23	3	0
Telocentric only	180	93	24	68	501	11	1	299	26	24	1

* Plants with one fragment had the normal diploid number of 14 chromosomes plus the telocentric chromosome; plants with both fragments had 13 normal chromosomes plus the telocentric chromosome and isochromosome.

progeny: (1) those with a normal complement of chromosomes, (2) those with a normal complement of chromosomes plus the telocentric chromosome, and, (3) those with 13 normal chromosomes plus the telocentric chromosome and isochromosome fragments (the parental type). Plants with the telocentric chromosome gave rise to only the first two types. No plant with the normal complement of chromosomes plus the isochromosome was observed, probably because gametes with seven normal chromosomes plus the isochromosome were inviable.

In plants with both fragments, the unpaired chromosome was almost invariably the isochromosome, which almost always appeared as a "doughnut," but occasionally opened up into a "rod." The fact (see below) that so few individuals in the progeny of plants with both fragments received the telocentric chromosome without the isochromosome indicated that the normal and the telocentric chromosomes were almost invariably paired at first meiotic metaphase and separated at first anaphase. In plants with the normal complement plus the telocentric chromosome, the fragment was almost always the unpaired chromosome, and appeared as a short "rod."

The unpaired chromosomes in plants with either one or both fragments frequently divided equationally at the first division and formed micronuclei at second telophase (occasionally at first telophase). In plants with both fragments, a few cases were observed in which the chromatids of the telocentric chromosome separated in the first division. Also, a few cases were observed in plants with the normal complement plus the telocentric chromosome in which the chromatids of a chromosome other than the telocentric one were separating at first anaphase.

The micronuclei in quartets of plants with the normal set of chromosomes plus the telocentric chromosome seemed to be almost invariably the same size. The frequencies of micronuclei are based on rather limited numbers of cells and are only approximate, because in some cases the micronuclei were probably not distinguished if they happened to lie over or under the macronucleus.

MACROSCOPIC OBSERVATIONS

Plants with 14 normal chromosomes plus the telocentric chromosome, or 13 normal chromosomes plus both fragments, were easily distinguishable macroscopically from each other and from normal plants. Both types were shorter in stature and had finer leaves and spikes than normals. Spikes of plants with both fragments were slightly more lax and had longer teeth on the outer glumes than spikes of normal plants. Spikes of plants with the telocentric chromosome were slightly more dense than spikes of normal plants, and lacked the elongation of the teeth on the outer glumes characteristic of plants with both fragments. Forty-eight plants with both fragments averaged 83 cm in height as compared with 98 cm for 109 normal sibs. The 48 averaged 14 culms each as compared with 13 for the 109 normals. Three plants with the telocentric chromosome averaged 78 cm and 19 culms.

TABLE 2

Fertility of sib plants with 14 normal chromosomes, 13 normal chromosomes plus 2 fragments, or 14 normal chromosomes plus the telocentric fragment.

STOCK	FLORETS	WITH SEEDS	WITHOUT SEEDS	FERTILITY	
		NUMBER	NUMBER	%	σ
Normal	Primary	275	11	96	1
	Secondary	30	260	10	2
Both fragments	Primary	231	68	77	2
	Secondary	8	291	3	1
One fragment	Primary	210	59	78	3
	Secondary	18	251	7	2

Plants with the telocentric or both fragments exhibited a considerable percentage of sterility, although especially vigorous plants were over 50 percent fertile (table 2). The sterility was probably due to a combination of causes: 1. In plants with both fragments, the fragments did not always go to

the same pole at first anaphase. When the isochromosome was not paired with the normal chromosome, presumably the fragments segregated at random with respect to each other. Occasionally the normal chromosome and the telocentric fragment apparently went to the same pole when both of the fragments were paired with the normal homolog. 2. Unpaired chromosomes (usually fragments) frequently divided equationally at the first meiotic division and formed micronuclei. 3. The duplication of chromatin in plants with either one or both fragments probably upset the physiological balance. There was no evidence as to which of the above factors was most important, except that in the plants with the telocentric chromosome it would appear that the third was the only one of importance.

TRANSMISSION OF THE FRAGMENTS

Data on the transmission of the fragments are presented in table 3. It is evident that the two fragments were rarely transmitted through the pollen,

TABLE 3

Transmission of the chromosomal fragments in diploid wheat.

PARENT PLANTS		CONSTITUTION OF THE PROGENY WITH RESPECT TO FRAGMENTS			
FEMALE	MALE	NO FRAGMENT NUMBER	2 FRAGMENTS NUMBER	1 FRAGMENT NUMBER	TOTAL NUMBER
Normal	2 fragments	102	1	0	103 (1)[1]
2 fragments	Normal	69	30	1	100 (15)
2 fragments	2 fragments	349	131	4	484 (41)

[1] The number in parentheses indicates the number of individuals examined cytologically.

and through considerably less than half of the eggs. No adequate data are available on the transmission of the telocentric chromosome from plants that had this fragment but did not have the isochromosome. However, the telocentric chromosome was occasionally transmitted without the isochromosome by plants that had them both.

EFFECT OF THE FRAGMENTS ON THE TRANSMISSION OF GENETIC FACTORS

The writer has established seven groups of linked genes in einkorn (1947b), though evidence is not conclusive that each group represents a different chromosome. Plants with one or more genes for mutant characters from six of these seven groups were crossed on plants with both fragments in order to test the effect of the fragments on the frequencies of the mutant characters in F_2. The results are presented in table 4. It is apparent that the transmission of the factors in only one of the six groups was affected. In this group (D) three factors were tested, and each of them was transmitted in very high frequency as compared with their transmission by normal plants. These results indicate that the chromosome containing the genes in this group was the one that had been broken.

The recessive genes for the three mutant characters that were affected in their transmissions were on the normal chromosome; the dominant alleles were on one or the other of the two fragments. Two causes for the effect of the fragments on the transmission of genes can be found in the cytological observations and in the data on transmission of the fragments. In the first place viable gametes with the two fragments were not formed as frequently as gametes with the normal complement of seven chromosomes. This was because

TABLE 4

Transmission of mutant factors by plants with both fragments.

LINKAGE GROUP (SEE FIG. 3)	PARENT PLANTS[1]	FACTOR SEGREGATING	CONSTITUTION OF PROGENY		
			TOTAL NUMBER	RECESSIVE PHENOTYPE	
				%	σ
A	Normal	gl-2	3,390	22	1
A	2 fragments	gl-2	398	19	2
A	Normal	c-1	3,920	26	1
A	2 fragments	c-1	143	27	4
C	Normal	e-2	3,683	21	1
C	2 fragments	e-2	48	27	6
C	Normal	yx-2	1,413	22	1
C	2 fragments	yx-2	257	24	3
D	Normal	c-2	1,160	26	1
D	2 fragments	c-2	35	77	7
D	Normal	cx-1	804	20	1
D	2 fragments	cx-1	404	64	2
D	Normal	y	3,090	25	1
D	2 fragments	y	1,108	66	1
E	Normal	Gp	2,832	28	1
E	2 fragments	Gp	42	21	6
F	Normal	G	1,434	26	1
F	2 fragments	G	106	25	4
G	Normal	e-1	5,227	15	0.5
G	2 fragments	e-1	42	19	6
G	Normal	ar-2	1,157	17	1
G	2 fragments	ar-2	109	19	4

[1] The normals were not sibs of the plants with fragments in all cases.

in about half of the sporocytes the isochromosome was not paired with the normal chromosome. As a result it would presumably segregate at random with respect to the other fragment. Moreover, when it was not paired with the normal chromosome, the isochromosome frequently was lost because it divided equationally at first anaphase. These two distributions would result in a considerable frequency of inviable daughter cells with only one fragment. In the second place gametes with the two fragments were not able to compete on equal terms with gametes possessing the normal complement of chromosomes. This was especially true with pollen where the two fragment chromosomes

were rarely transmitted, probably because the isochromosome had a duplication of a whole arm of a chromosome.

So far data have not been obtained on the effect of the telocentric fragment alone on the transmission of factors in the affected linkage group. Such data

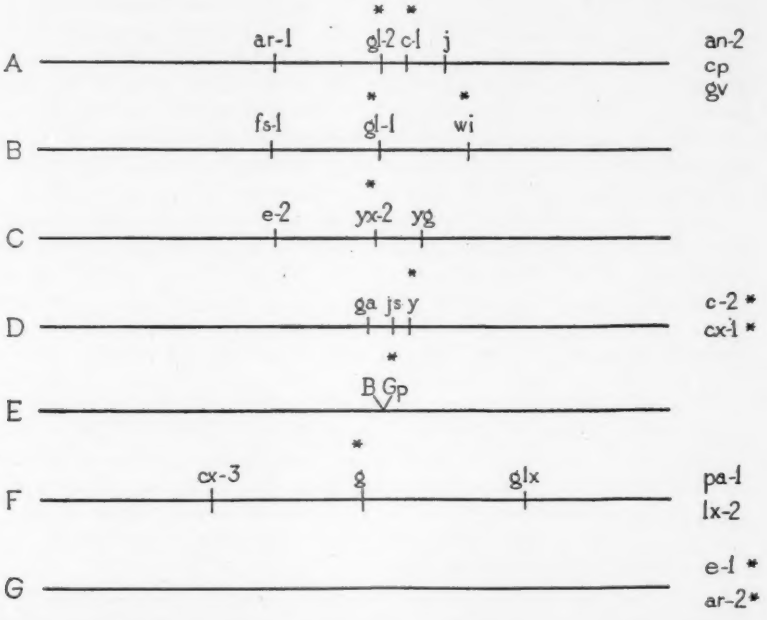

FIGURE 3.—A linkage map of the factors known to be linked in einkorn. The evidence for linkage of *e-1* and *ar-2* is indirect (SMITH 1947b). (See SMITH 1939, for descriptions of the mutant characters.) One inch represents approximately 15 crossover units. The orders of some of the factors on the chart, as well as all the factors on the right-hand margin, were not definitely established. The groups of linked factors are not necessarily on different chromosomes. However, one or more factors in each group were tested with factors in each of the other groups, and found to be apparently unlinked. An asterisk indicates the factors tested for abnormal segregation in plants with the fragments.

would indicate in which half of the chromosome each of the genes was located (if the "telocentric" chromosome really has a terminal centromere).

DISCUSSION

Fragments such as those described in this paper have been known for a number of years in Datura (BLAKESLEE and AVERY 1938), maize (RHOADES 1940), and a few other species. The reader is referred to the paper by RHOADES for an excellent review of the literature up to that time, and for a thorough

cytogenetic study of a telocentric chromosome in maize. It is more unusual that the two components of a chromosome apparently broken at the centromere, were recovered.

The writer wishes to emphasize the usefulness of such fragments in the study of linkage relations of genes. In a number of respects these fragments are more useful than trisomes for this purpose. A plant with such fragments is probably more vigorous and fertile than a plant trisomic for the same chromosome would be. Also, in testing for disease resistance, winter hardiness, etc., the plants with the normal complement of chromosomes would be the ones recovered in high frequency, so the significance of the tests would depend primarily on the response of normals rather than on the weak plants with the fragments. Moreover, it should be possible to localize the genes in the two arms of the chromosome as has been done in a few other species (see RHOADES).

Another important use of the fragments was suggested by SMITH (1946). By means of an interchange any other of the seven haploid chromosomes could be associated with one of the fragments. As a result, any gene in either of the two affected pairs of chromosomes would be altered in transmission (unless the percentage of crossing over between the breaks and the gene approached 50 percent). Thus, as with the deficiency-duplication complex reported by SMITH (1947b), with five selected interchanges it would be possible in this or any other seven chromosome species to build up five stocks that could be used to determine the linkage relations of genes much more efficiently than by the usual method of using genes alone, because linkage tests could be made merely by determining the effects of the chromosomes, taken two at a time, on the transmission of the genes. After it was determined in which chromosome a gene lay, its position with relation to the rest of the genes in that chromosome could be established in the usual manner.

If chromosomal aberrations affecting three different chromosomes were available (the writer now has two in this diploid wheat), the same results could be accomplished with three interchanges—and, hence, three stocks would suffice (again barring complications due to the fact that some factors might be 50 or more crossover units from the breaks) to locate genes in all seven chromosomes.

SUMMARY

Apparently X-rays broke a chromosome in the centromere, producing two telocentric fragments, one of which later formed a secondary trisome. Plants with 14 normal chromosomes plus the telocentric chromosome and plants with 13 normal chromosomes plus the two fragments were fairly vigorous and fertile but were distinguishable from each other and from normals on the basis of macroscopic as well as cytologic characteristics.

The fragments were rarely transmitted through the pollen and through less than half of the eggs.

Genes from six of seven linkage groups were tested for abnormal transmission by plants with 13 normal chromosomes plus the two fragments. Genes

from five linkage groups were inherited in about the same frequency from plants with the fragments as from normal plants. However, three genes from one linkage group were transmitted to a very high proportion of the progeny of plants with the fragments. Thus the fragments appear to be useful tools in determining whether genes are located in this particular chromosome.

A method is suggested for associating the fragments with other chromosomes (one at a time) by means of interchanges. The reduced transmission of the fragments and the interchanged chromosomes could be used to determine whether genes whose loci were unknown were located in either of the two chromosomes. Five stocks with the fragments associated in each stock with a different chromosome of the genom by means of interchanges could be used to test the locations of genes in any of the seven chromosomes of the species.

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EFFECT OF THE DE17 ALLELE ON DEVELOPMENT OF THE MAIZE CARYOPSIS

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University of Wisconsin, Madison, Wisconsin

Received March 12, 1947

MOST of the defective seed types which come to light in maize populations subjected to self-fertilization are lethal. Search discloses an occasional one, however, which may be propagated in homozygous condition. The present study deals with the developmental effects of a gene of the latter kind. A pronounced deleterious influence is exerted on the seed but not upon the ensuing plant. The investigation was undertaken to determine the manner in which the gene acts to impair seed development.

MATERIAL

The parent ear from which the defective stock was derived was borne upon a hybrid plant in a population obtained by pollinating the variety Western Plowman by an inbred strain known as Indiana Kd 104. We are indebted to DR. N. P. NEAL for the material. The recessive defective allele may have originated as a recent mutation in the inbred line but it is much more probable that it was present in the Western Plowman parent. The parent ear had been self-fertilized and bore 441 normal and 147 defective seeds. This is an exact three to one ratio. Subsequent tests disclosed the presence of the same, or a similar, defective allele in an unrelated family derived from a self-pollinated plant in the Minnesota 13 variety. The original self-pollinated ear in this case bore 199 normal and 120 defective seeds. This ratio departs widely from expectation on the simple Mendelian basis, the normal class being low. Since these defective seeds were relatively thin and papery, the Minnesota 13 stock was not used further. It is proposed to designate this allele for defective seed *de17* in accordance with established usage (EMERSON, BEADLE and FRASER 1935).

The simple recessive behavior of the defective character is borne out by the data in tables 1 and 2. Pollination of four heterozygous plants with pollen from homozygous defectives gave equal numbers of normal and defective kernels within the limits of random sampling (table 1). Self-pollination of heterozygotes gave a close approximation to the expected 25 percent of defectives (table 2). The kernels at the tips of these ears were discarded before the counts were made in order to reduce the likelihood of including parthenocarpic seeds in the defective class. It may be concluded from the breeding data that the defective allele is transmitted through the male and female gametophytes in normal Mendelian proportions.

An important advantage of using a defective which can be propagated in

Paper from the DEPARTMENT OF GENETICS, AGRICULTURAL EXPERIMENT STATION, UNIVERSITY OF WISCONSIN, Madison, No. 367.

homozygous condition in a histological study is that material of known genetic composition can be examined at the stages of development through which the seed passes before it is classifiable on the cob as defective or non-defective. It is clear from observations on seed development following interspecific hy-

TABLE 1

Backcross ratios from De17de17 ♀ × de17de17 ♂ matings.

PED. NO.	NUMBER OF SEEDS	PERCENT DEFECTIVE
T10-7×*de17*	477	54.5
-10×*de17*	530	50.4
R10×R11A	457	50.1
R11×R10A	703	51.8
Total and Average	2,167	51.7

TABLE 2

F_2 ratios from De17de17 plants selfed.

PED. NO.	NUMBER OF SEEDS	PERCENT DEFECTIVE
R1-1	699	28.3
-2	684	25.1
-3	645	26.0
R5-1	444	22.1
-2	452	24.7
-3	291	26.1
R6-1	295	28.5
R8-1	443	24.8
-2	725	23.2
-3	579	23.0
Total and Average	5,257	25.05

bridization that the changes which occur during the early post-fertilization period are critically important for the subsequent course of growth (compare BRINK and COOPER 1947). The defective used in the present investigation cannot be recognized on segregating ears with certainty until about ten days after pollination. All the material for histological study was collected, therefore, from ears borne by homozygous defective plants. The two classes of seeds were obtained on different ears through the application of pollen from defective and homozygous normal individuals, respectively.

No departure from the regular type of seed development can be detected in kernels whose endosperms and embryos carry the normal allele of *de17* in single dose. Weights of the normal kernels borne on the ears from three *De17 de17* plants crossed with *de17de17* were compared with those of normal kernels obtained by pollinating three closely related *de17de17* individuals with

pollen from a *De17de17* stock. The average weights of the two groups of *De17* kernels were almost identical. That is to say, kernels of normal phenotype develop as well on *de17* as on *De17* plants.

MATURE WEIGHT OF THE DEFECTIVE KERNELS

The weight of the mature defective caryopsis in the line in which the allele was first discovered was approximately 25 percent, on the average, of that of the normal kernels on the same ears. This is shown by the data in table 3. The

TABLE 3

Weight in grams of normal and defective seeds on segregating ears.

EAR NO.	NORMAL		DEFECTIVE	
	NO. SEEDS	AVGE. WEIGHT	NO. SEEDS	AVGE. WEIGHT
7787	100	.327	26	.103
7787	100	.321	21	.149
7788	101	.315	24	.011
7910	100	.347	39	.089
7912	101	.332	29	.042
7915	101	.302	36	.124
7915	100	.278	32	.072
7920	101	.322	41	.098
7923	100	.352	30	.069
7924	100	.332	37	.028
7926	101	.332	39	.112
7929	100	.300	38	.150
7951	100	.366	28	.031
7957	100	.337	28	.161
7965	100	.338	23	.037
7979	100	.349	33	.029
Total		5.250		1.305
Average		.328		.082

defectives when extracted from outcrosses subsequently made to various unrelated lines, however, regularly proved to be relatively lighter in weight than in the original stock. The weights on several such ears were only 12 to 15 percent of the values for the respective normal sib kernels. The decreased development of the extracted defective seeds is doubtless a result of changes in the residual inheritance. Although few outcrosses were tested it appears reasonably certain that this defective lies relatively low on the scale which MANGELSDORF (1926) used in classifying heritable maize variations of this kind.

THE STARCH GRAINS IN DEFECTIVE SEEDS

The amount of starch stored in the endosperm of defective seeds is much less, of course, than in non-defective kernels. The starch grains present in

defective seeds, however, are nearly normal in size and form, and give the usual reaction with iodine.

GERMINATION AND SEEDLING ESTABLISHMENT

Seed from five segregating ears, free of disease, on which the defective kernels were relatively well developed were planted in sand in the green house. Counts made 15 days later showed that the percentages of seedlings emerging

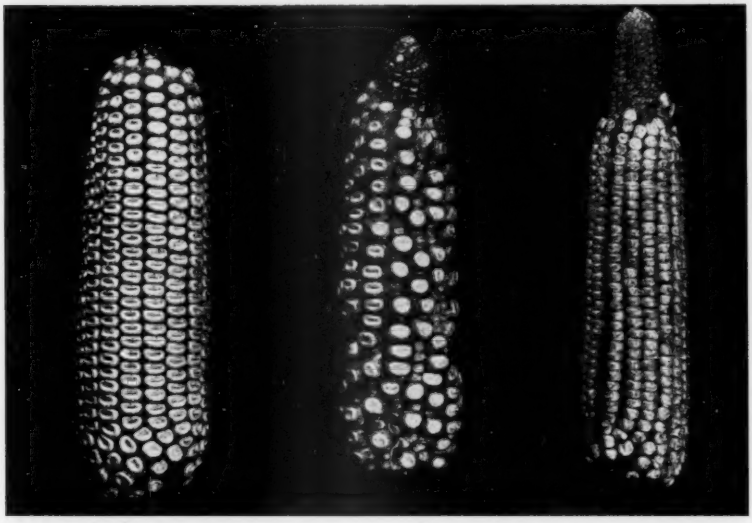


FIGURE 1.—An ear from a homozygous defective plant selfed is shown at the right. The middle ear is the result of backcrossing a heterozygous defective to the defective type. The ear at the left is normal.

from the normal seeds were 96, 99, 98, 100 and 99, respectively. The corresponding values for the defective kernels were 52, 50, 36, 58 and 31 percent. Many of the seedlings from the defective kernels were very small and abnormal in form. Most of these weak individuals die even under favorable conditions for growth. A small proportion of the kernels from many ears, however, give rise to thrifty but undersized seedlings.

The more vigorous homozygous defective seedlings after transplanting to the field grow into vigorous plants. When full grown the latter are about one foot shorter than their normal sibs. Ear shoots and tassels are well developed on the defectives. Pollen is formed abundantly and full complements of defective seed are readily obtained on selfing. Representative normal, defective and segregating ears of the original stock in which the defective character was found are shown in figure 1.

GROWTH OF DEFECTIVE AND NORMAL KERNELS

Data on the growth of defective and normal kernels were obtained from a single family of *de*17 plants. The two classes of kernels were obtained by pollinating alternate individuals in a row with *De*17 and *de*17 pollen, respectively. Collections were made at 0, 6, 9, 12, 16, 24 and 32 days after pollination. The fresh kernels were removed from the cobs and the glumes and projecting vascular tissue were cut off with a sharp scalpel. The caryopses were then dried in a current of warm air. The kernels were allowed to stand for two days at room temperature before counting and weighing. The data are summarized in table 4 and shown graphically in figure 2.

TABLE 4

Average weight in grams of normal and defective kernels up to 32 days after pollination.

DAYS	NORMAL KERNELS			DEFECTIVE KERNELS		
	NO. OF EARS	NO. OF KERNELS	AVGE. WT.	NO. OF EARS	NO. OF KERNELS	AVGE. WT.
0	—	—	—	4	251	.0017
6	6	414	.0045	6	390	.0044
9	8	532	.0083	8	544	.0086
12	12	789	.0172	12	818	.0132
16	8	539	.0461	8	533	.0241
24	8	548	.1107	8	538	.0385
32	8	543	.1774	8	549	.0402

No difference is observable in the dry weights of defective and normal kernels at six and nine days after pollination although both classes of caryopses increase their substance about five-fold over this period. The normal kernels, on the average, are about 30 percent heavier than the defectives at 12 days. The difference increases to 90 percent at 16 days. The average weights for the eight respective samples of defective kernels no longer overlap those of the normal kernels at this time. The defective caryopses show a further increase in dry weight at 24 days, but little change occurs beyond this time. As the defective kernels approach their maximum weight at 24-32 days the normal kernels are three to four times as heavy. Weights were not taken beyond 32 days, although the normal kernels continue growth.

CHANGES IN THE ENDOSPERM, EMBRYO AND PLACENTAL REGION AT SIX TO TEN DAYS

The above data on dry weight indicate that the defective and normal caryopses begin to differentiate from each other in total growth prior to 12 days. In searching for the basis of this differentiation an attempt was made to estimate the size of the endosperm and embryo at six, eight and ten days, and to

characterize the placenta at these times in the two classes of kernels. The term "placenta," as here used, is intended to apply to the saucer-shaped region extending from the nucellus and basal margin of the endosperm to the terminus of the main vascular element of the spikelet. Two groups of four well-developed plants, bearing all defective and all heterozygous normal seeds, respectively, were chosen in the family from which the weight data presented

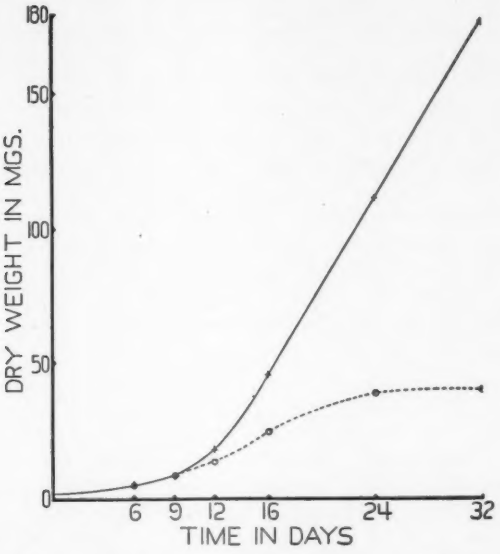


FIGURE 2.—The dry weight of normal (solid line) and defective (broken line) caryopses up to 32 days after pollination.

in tabie 4 were taken. Successive collections of portions of the ear were made from these plants at six, eight and ten days. The distal one-third of each ear was discarded at the time the first samples were taken, and the basal parts of the ears were not used. The kernels taken for examination, therefore, came from the relatively homogeneous mid-sections of the ears.

The material was fixed in Carnoy's and Karpechenko's solutions and preserved in 70 percent alcohol. The caryopses were dehydrated, imbedded in paraffin, mounted and cut on the microtome so as to obtain median longitudinal sections passing through the silk attachment region and the embryo, bisecting the latter symmetrically. After staining in Delafield's haematoxylin the numbers of cells in endosperm and embryo in these median sections were estimated by counting nuclei under a microscope fitted with a 10X ocular, an 8 mm objective, and a net micrometer.

During early growth of the maize caryopsis nearly all of the nucellus is

digested, the contents of the cells presumably being absorbed by the rapidly expanding endosperm. Attempts were made to measure the rate at which the nucellus disintegrates in normal and defective seeds. A satisfactory way of doing this was not found since the structure, including the cell walls breaks

TABLE 5

Average number of nucleated cells in endosperm, embryo and the placental region in median sections of the caryopsis at 6, 8 and 10 days.

NORMAL					DEFECTIVE				
PLANT NO.	NO. OF CARY-OPSES	AVGE. NO. OF NUCLEATED CELLS			PLANT NO.	NO. OF CARY-OPSES	AVGE. NO. OF NUCLEATED CELLS		
		ENDOSP.	EMBRYO	PLA-CENTAL REGION			ENDOSP.	EMBRYO	PLA-CENTAL REGION
				6-days					
A10	8	335	50	265	A9	2	321	44	272
A12	4	384	93	154	A11	2	368	75	202
A14	7	429	99	243	A13	6	375	105	275
A16	7	372	66	161	A15	6	371	71	258
Total & Avge.	26	380	77	206	Total & Avge.	16	359	74	252
				8-days					
A10	9	889	228	162	A9	5	834	172	131
A12	4	815	433	76	A11	10	1,027	490	133
A14	7	977	387	144	A13	10	842	408	217
A16	0	—	—	—	A15	7	936	290	203
Total & Avge.	20	894	349	127	Total & Avge.	32	910	340	171
				10-days					
A10	7	1,679	719	86	A9	8	1,181	524	143
A12	6	1,591	824	36	A11	10	1,442	1,085	121
A14	7	1,710	1,104	92	A13	9	1,415	928	160
A16	8	1,432	717	52	A15	8	1,521	985	192
Total & Avge.	28	1,603	841	66	Total & Avge.	35	1,390	880	154

down in a rather diffuse manner. The visual evidence is clear, however, that the nucellus in a defective seed is somewhat more persistent than in a normal seed.

The cellular changes in the placental region of the kernel can be measured in the median sections. As in the nucellus, the contents of the cells of that part of the placenta adjacent to the endosperm are progressively absorbed by the

actively growing endosperm. The cell walls tend to persist, however, leaving the framework of the tissue more or less intact after nucleus and cytoplasm have disappeared. This makes it possible to estimate the number of nucleated and non-nucleated cells in this region as regression of the tissue proceeds. The number of stainable nuclei was counted in a band of the placenta .225 mm (five micrometer divisions) wide bounded on one end by the endosperm and at the other by the massive disc-shaped structure in which the principal vascular elements of the caryopsis terminates. The area chosen for the counting lay in the uniform mid-portion of the placenta. All the stained nuclei were recorded in the area thus circumscribed.

The data on number of endosperm and embryo nuclei and on number of nucleated cells in the designated portion of the nucellus in median sections of normal and defective seeds are summarized in table 5. It is apparent from these data that the period from six to ten days is one of rapid growth of the endosperm and embryo. The latter increases in size by about the same amount in both normal and defective seeds. This relation appears to hold for the endosperm also at six and eight days. The estimated average number of cells in median section of the normal seed at ten days is 1603. The corresponding value for the defective seed is 1390. The data are too few to establish the significance of this difference but they suggest that the defective endosperm has begun to lag behind the normal. Two other facts point to the conclusion that at ten days the endosperms in the two classes of seeds are different. The fresh seeds collected at this time are unlike in texture, the normals being firmer. The difference is less perceptible in fixed material due to hardening of the pericarp. The endosperm in the ten day old defective seed is also more subject to shrinkage on fixation than that of the normal.

The evidence shows that the cells in the placental region are depleted of their contents more rapidly in normal than in defective seeds. At six days there are about 22 percent more cells containing stainable nuclei in the designated placental area in defective than in normal seeds. The difference increases to 35 percent and 133 percent, respectively, at eight and ten days. It appears probable that better development of the endosperm in normal seeds and the more rapid depletion of the adjacent placenta are associated phenomena.

DEVELOPMENT OF THE CARYOPSIS

A detailed histological study of the normal and defective kernels was undertaken using material collected at intervals of 18 and 28 hours, two, four, six, eight, nine, ten, 12, 14 and 18 days after pollination. The two classes of seeds again were obtained by applying pollen from homozygous normal and defective plants to ear shoots on *de*17 individuals. The kernels were fixed in Carnoy's and Karpechenko's solutions, imbedded in paraffin, sectioned at 12 micra and, for the most part, stained in Delafield's haematoxylin. Various combinations of stains were used on material collected at the critical stages of development, namely, six, eight and ten days, in order to bring out the relationships existing between the different parts of the developing caryopsis.

THE NORMAL KERNEL

The ovary is nearly spherical at the time of fertilization, being somewhat flattened on its upper surface immediately below the region of the silk attachment (figure 3). The ovule is oriented within the ovary in such a manner as to bring the female gametophyte (F) immediately beneath this flattened face The vascular tissue which enters the pistillate spikelet is profusely branched a short distance below the ovule. The branches, barring three, are entwined and terminate in the ovary wall opposite the base of the ovule so as to form a disk of vascular tissue (VD) across the stalk of the spikelet. Two branches on the upper side extend the full length of the ovary and out into the silk. A third branch on the lower side extends through the ovary wall to the region of the stylar canal.

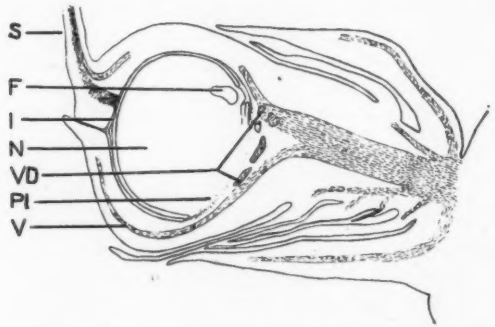


FIGURE 3.—Longitudinal section of mature ovary. S, silk; F, female gametophyte; I, integuments; N, nucellus, VD, vascular disk; Pl, placenta; V, vascular bundle. ×24

No vascular elements enter the ovule. Conduction from the vascular tissue is by means of the storage and parenchymatous tissue present in the placental region (Pl). The cells of the stalk just beyond the vascular disk are small and densely cytoplasmic and later (four to five days after fertilization) become packed with storage materials. The thin-walled parenchymatous cells distal to the storage region are large and highly vacuolate with prominent nuclei. These cells are similar in size and shape to those of the nucellus (N) with which they merge. There is no definite line of demarcation between the cells of the two regions.

The amphianatropous ovule virtually fills the ovarian cavity. It is unlike any of the standard forms being intermediate between the amphitropous and anatropous types. The female gametophyte occupies a relatively small portion of the nucellus. It is oriented so that its longitudinal axis coincides with a line drawn from the base of the silk (S) to the micropyle.

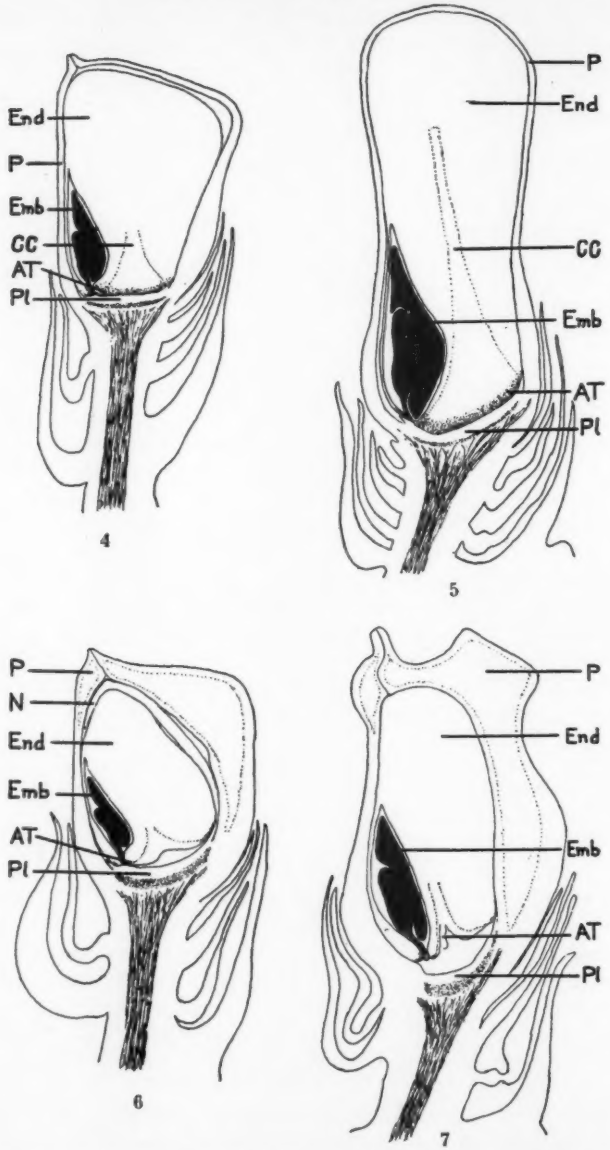
Fertilization was found to occur between 16 and 18 hours after pollination and the entire ovary is then stimulated to rapid growth. The primary endosperm nucleus divides shortly after fertilization and mitoses occur in rapid

sequence so that endosperms with eight and 16 nuclei accompanied by a zygote or two-celled proembryo are present in material collected ten hours later. The endosperm grows first at the expense of the enlarging cells of the nucellus. Later it absorbs nutrients from the placental region.

The rate of growth of the endosperm is such that the space occupied by it and the accompanying embryo in material collected four days after pollination is at least 20 times the size of that occupied by the mature female gametophyte. The growth of the endosperm is toward the antipodals; and at the base it expands into the nucellus at right angles to the longitudinal axis of the ovary so that an inverted, irregularly cone-shaped structure is formed which is flattened on the germinal face. This flattened face is approximately at right angles to the base of the endosperm. The endosperm continues to grow at least partly at the expense of the nucellus and maintains the same general shape for the first eight or nine days of development. The base of the endosperm has, by that time, spread out so that its periphery has reached the outer limits of the nucellus. Thereafter the endosperm quickly consumes the remaining nucellus and has assumed the shape of the ovule in material collected at the 12-day period (figure 4). During these early stages of development the cells are generally rather than locally meristematic. Later the peripheral layers remain meristematic and the central cells enlarge and elongate. Storage of starch begins about 12 days after pollination. The initial storage is in the large cells near the apex of the kernel.

The epidermal cells of the endosperm remain relatively undifferentiated during the first four or five days of development. Thereafter the cells on the basal surface of the endosperm facing the placental region become transformed into absorbing cells which function in the transfer of nutrients from the maternal tissue to the growing endosperm. This transformation is initiated in the cells immediately adjacent to the base of the embryo and gradually spreads from that point so that in material collected 12 days after pollination all of the basal epidermal cells have become converted into an absorbing tissue (AT). In the course of this transformation each cell becomes much elongated and its nucleus moves to the end of the cell toward the main body of the endosperm. The walls on the basal surfaces of these cells become much thickened and such thickening extends along their lateral faces. This thickening of the lateral walls gradually diminishes toward the apices so that the upper portions and the upper faces are thin walled (figure 9). The fibrillar appearance of the cytoplasm in fixed material is due to the fact that longitudinal folds of the cytoplasm extend into these thickened walls to the primary wall of the cell (figure 10). The basal layer remains in close association with the cells of the placenta even in poorly fixed kernels. If shrinkage occurs there is a break across the endosperm above the differentiated cells of the absorbing layer.

The transformation of the basal epidermal cells into an absorbing tissue has an immediate effect on the adjacent layers (ten to 12) of parenchymatous cells in the placenta. The protoplasm in the superficial placental cells becomes disorganized and the nuclei disappear even though the cells remain turgid. The breakdown of the protoplasm in the cells of this tissue advances across the



FIGURES 4 to 7.—Longitudinal sections of developing kernels. Fig. 4. Normal kernel, 12 days after pollination. Fig. 5. Same, 18 days after pollination. Fig. 6. Defective kernel, 12 days after pollination. Fig. 7. Same, 18 days after pollination. P, pericarp; End, endosperm; CC, conducting cells of endosperm; Emb, embryo; AT, absorbing tissue of endosperm; Pl, placenta. ×8

placenta in conjunction with the transformation of the adjacent epidermal cells of the endosperm into a specialized tissue. The breakdown extends eventually into the middle layers of placental tissue so that a cup of cells with disorganized contents comes to lie below the endosperm and opposite the apex of the vascular bundles although not extending to the latter.

The first evidences of the disorganization of the protoplasm in the parenchymatous cells of the placenta are to be found in young kernels collected six days following pollination. A group of cells with disorganized protoplasm is present immediately opposite the region of the embryo. It extends into the placenta. The surface cells of the latter tissue show evidences of a loss of some of the materials stored therein indicating a movement of these materials toward the actively absorbing region of the endosperm. As growth proceeds these storage materials continue to move toward the endosperm and thereafter the proximal portion of the placenta serves as a region for temporary storage and transfer of nutrients from the vascular system to the endosperm. The parenchymatous cells in the placental region and even the deeper lying storage cells of the placenta become more or less flattened as the endosperm continues to grow and expand.

Further cells at the base of the endosperm become transformed into elongate absorbing cells so that three or four layers of such cells are present in 18-day old kernels (figures 5 and 8a). A central core (CC) of large, elongate cells extends from the base of the kernel toward the apex. This core acts as a conducting tissue and transfers nutrients from the absorbing region to the area of storage and later also serves as a place of storage (WEATHERWAX 1930, LAMPE 1931).

The peripheral layers of cells other than those in the absorbing region remain in a meristematic condition until some 20 or 25 days after pollination (FISK 1927). The epidermal layer consists of cells that are small and densely cytoplasmic. This epidermal layer becomes the aleurone layer in the mature kernel.

THE DEFECTIVE KERNEL

All of the defective kernels have badly shrunken endosperms and poorly developed embryos at maturity. The usual interval (16 to 18 hours) elapses between pollination and fertilization and early development of the kernel proceeds at the normal rate. Zygotes and two-celled proembryos accompanied by endosperms with eight or 16 nuclei are present in samples collected 28 hours after pollination.

The development continues at a normal rate up to six days after pollination at which time a cone-shaped endosperm is present. Thereafter growth slows down so that the base of the endosperm does not reach the periphery of the ovule until 12 days after pollination (figure 6). A large mass of the nucellus (N) is still present near the apex of the ovule at this interval. This nucellar tissue is consumed during the next few days so that little, if any, remains at 18 days (figure 7).

The cells of the basal epidermal layer of the endosperm are densely cytoplasmic in the material collected at the six day interval. These cells do not

elongate and become thick walled and as a result they do not acquire the fibrillar appearance characteristic of this tissue in the normal kernels at later stages of development. Rather they enlarge and become highly vacuolate (figures 11 and 12). There is a close relationship between them and the parenchymatous tissue of the placenta in well-fixed material. When differential shrinkage occurs due to fixation, the endosperm pulls away from the surrounding maternal tissue leaving a wide gap between the basal portion of the endosperm and the placenta.

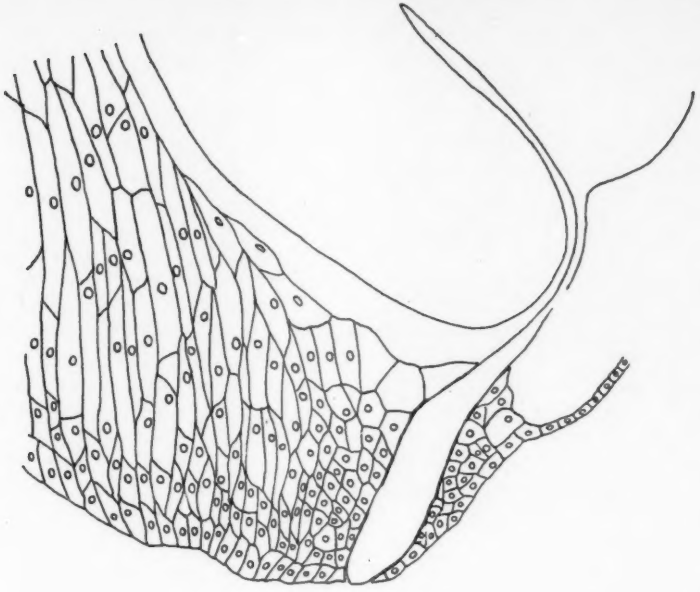
The protoplasm of the cells of the central portion of the basal epidermal layer which acts as an absorbing tissue in the normal kernels begins to break down in the defective kernel at ten to 12 days after pollination (figure 11). The breakdown spreads to the adjoining cells both of epidermal layer and the adjacent endosperm so that the basal layers of cells of 18-day endosperms are, for the most part, empty and badly shrunken (figures 7 and 8b). A collapse of the central core of the endosperm accompanies this breakdown and the endosperm is unable to obtain the requisite supply of nutrients for normal development.

The effect of the basal epidermal cells on the placenta is quite different from that in the normally developing kernel. The adjacent parenchymatous cells increase in size and in many the cytoplasmic content becomes shrunken about stainable nuclei in 12-day material. Other cells lose their entire content. They continue to expand and in 18-day material many of the cells immediately adjacent to the endosperm have ruptured. The deeper lying cells of the placenta tend to retain their storage materials.

The embryos develop at the normal rate for the first six days after pollination and then growth appears to slow down slightly. The lag in embryo development was noted in the samples collected at each interval thereafter. The embryo continues to grow at the expense of an undernourished endosperm so that much of the endosperm is empty and badly collapsed at 18 days.

DISCUSSION

The differentials conditioning normal and defective seed development in the present study are the *De*17 and *de*17 alleles, respectively. The effect of the recessive allele in homozygous condition is to reduce kernel weight to 25 percent or less of the standard value. Homozygous defective embryos are potentially capable of growing into thrifty, fertile plants only a little shorter than their normal sibs. Special care must be taken, however, in propagating *de*17 *de*17 plants at the early stages because even the most vigorous seedlings of this class initially are weak. Homozygous defective plants to which normal pollen is applied develop normal seeds. This makes it possible to obtain phenotypically normal and defective kernels at will by controlling the pollination of *de*17 plants. It is evident from this fact, also, that the difference in seed development is attributable to the composition of the endosperm or embryo, or both and is independent of the genetic make-up of the pistillate parent in



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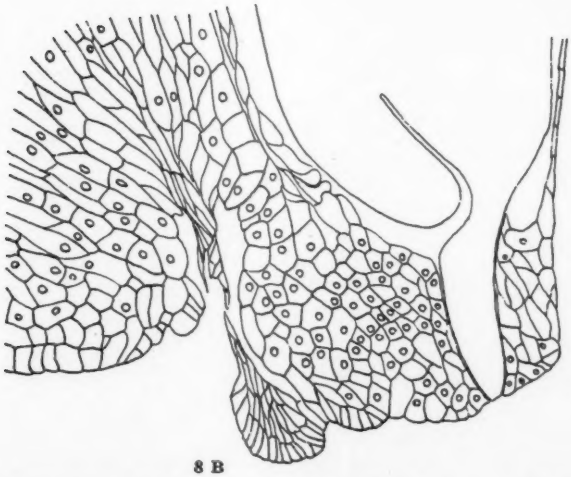
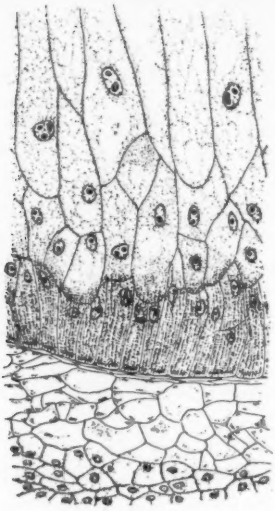


8 B

FIGURE 8.—Basal portion of endosperms, 18 days after pollination. A, Normal. B. Defective. ×80



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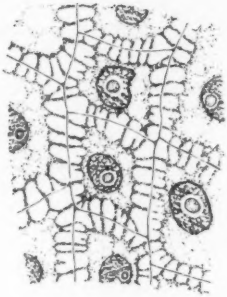

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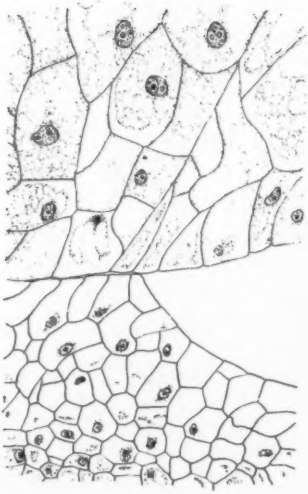

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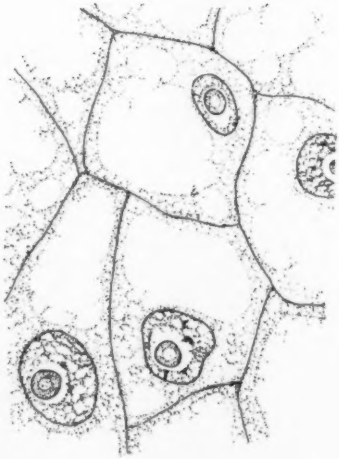

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FIGURES 9–12

respect to the *De*17 and *de*17 alleles. Both endosperm and embryo are markedly underdeveloped in the mature defective caryopsis.

Both normal and defective caryopses increase in weight approxima ely five-fold during the first nine days after pollination. The distribution of the dry weight increases as between endosperm and embryo and the pericarp was not determined. Much of it probably occurs in the pericarp. The embryo remains a comparatively small structure during this period: and endosperm growth involves displacement of the nucellus, the substance of the latter tissue being rapidly consumed in the process. At 12 days the defective kernels have fallen slightly behind the normals in dry weight. The difference is much larger at 16 days, and continues to increase rapidly up to 24 days beyond which time the defectives make little growth.

The histological studies reveal a relationship between the initial divergence in weight of the two classes of kernels and the differentiation of an absorbing region in the endosperm. During the interval between six and 12 days the cells on the basal surface of the endosperm facing the placental region in normal kernels become elongated, the nuclei move to the inner end of the cells, and the cytoplasm assumes a dense, fibrillar appearance. These structural changes indicate that the basal cells become highly active at this time in absorbing nutritive materials from the placental region and transmitting them to the more distal portions of the endosperm. The basal cells of the endosperm in defective seeds do not become similarly transformed into absorbing elements. Rather they enlarge about equally in all dimensions and become highly vacuolate. Failure of the defective endosperm to differentiate a basal absorbing tissue during the interval between six and 12 days explains the limited growth which these kernels subsequently make. A few days later the cells in this region of the defective endosperm begin to break down. Eventually many cells in the basal area and in the adjoining central region of the endosperm collapse thus becoming entirely nonfunctional in the absorption of nutrients into the seed.

There is some evidence of a lag in growth of the defective endosperm paralleling the formation of the absorbing layer in the endosperm of normal seeds. The data in table 5 relating to eight- and ten-day endosperms suggest this. Furthermore, the expanding base of the young endosperm reaches the outer limit of the nucellus about three days earlier in normal than in defective seeds.

The changes which appear during the first 12 days in the placental tissues of the two classes of caryopses are in conformity with the behavior of the re-

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FIGURE 9.—Longitudinal section through portion of absorbing tissue of endosperm of normal kernel showing fibrillar appearance of cytoplasm and thick walls of epidermal layer, 12 days after pollination. ×210

FIGURE 10.—Transverse section of same showing folds of cytoplasm in the thickened walls of the epidermal cells. ×920

FIGURE 11.—Longitudinal section through portion of absorbing tissue of endosperm of defective kernel showing thin-walled epidermal layer, 12 days after pollination. ×210

FIGURE 12.—Transverse section of same showing large, highly-vacuolate, thin-walled cells of the epidermal layer. ×920

spective associated endosperms. The parenchymatous cells of the placenta are quickly and extensively depleted of their total contents by the regularly differentiating normal endosperm. The placental cells in kernels possessing defective endosperms are more slowly and less completely depleted. The difference appears to be a direct function of the absorptive capacities of the normal and defective endosperms.

Miniature seed (*mn*) in maize which is similar to *de*17 in that it gives vigorous and fertile adult plants even though the caryopsis is greatly reduced in size has been studied recently by LOWE and NELSON (1946). These investigators conclude that the principal factor operating to restrict development of the miniature seed is a break in cellular contact along the interface between the endosperm and the placenta. In sectioned preparations the two tissues at 14 days are loosely connected and at 17 days are completely separated in miniature kernels. We have made the same observation repeatedly in *de*17 caryopses. The phenomenon appears to us to be due mainly, however, to the pronounced shrinkage of the defective endosperms on fixation. It is evident that with the eventual disorganization and breakdown of the absorbing layer in the endosperm of defective kernels physical continuity between the endosperm and the chalazal region will be interrupted. We interpret this rupture as a direct consequence of the collapse of the outer or basal endosperm cells rather than as a result of a shrinkage of the chalazal tissue due to some prior influence of the defective endosperm on the cells in this region as LOWE and NELSON suggest.

It seems important to point out in this connection that the outer layers of cells in the chalazal region of both normal and defective seeds become moribund very quickly as the young endosperm expands. The protoplasts are digested and their products are presumably absorbed by the endosperm. The cell walls are more persistent than those of the nucellus, however, where a similar process occurs in the juvenile seed. Data in table 5 show that depletion of the chalazal cells occurs more rather than less rapidly in the normal caryopses. The significant fact, however, is that in both normal and defective kernels this region of the chalaza becomes physiologically inactive due to destruction of the protoplasts. The two classes of caryopses are alike in showing a break in the continuity of living cells.

Functionally, this region in the maize caryopsis becomes a chalazal pocket (compare COOPER and BRINK 1940) into which nutrient materials are moved from the vascular tissue below and from which they are absorbed by the endosperm. It is probable that the nutrients are present in relatively soluble form and that they are ordinarily lost in the preparation of stained sections. Occasionally, however, we have seen massive amounts of dense material, homogeneous in appearance, lying between the absorbing cells of the young endosperm and the adjacent framework of cell walls in the placenta. Such deposits have been observed only in normal kernels. It is likely that they are accumulated less abundantly in association with defective endosperms and more completely dissipated in processing the tissue.

The available data do not show whether the *de17* gene exerts a direct parallel influence on the endosperm and embryo or acts directly on the endosperm only. The severely restricted development of the defective endosperm in itself is sufficient to account for the failure of many of the associated embryos to reach a viable condition and for the others to yield weak seedlings. The somewhat shorter stature of adult *de17de17* plants, as compared with their normal sibs may be due either to the handicap incurred at the seedling stage because of poor seed development or to this factor plus a continuing but only mildly deleterious effect of the *de17* allele on later growth.

SUMMARY

The study is concerned with the effect on development of the maize caryopsis of the recessive defective -17 (*de17*) allele.

The *de17* allele in homozygous condition reduces the weight of the mature kernel to 25 percent or less of the normal value. The embryos in many *de17* seeds are viable, however, and produce seedlings which, on overcoming the initial handicap of small size, may grow into vigorous, fertile plants. Mature *de17* plants are about one foot shorter than their *De17* sibs.

De17 kernels develop as well on homozygous *de17* plants as on normal individuals. Comparable samples of the two classes of caryopses, *De17* and *de17*, may be obtained at all stages of development, therefore, following the appropriate pollinations on *de17* plants.

Defective and normal kernels increase in weight at the same rate up to nine days after pollination. Thereafter the *de17* kernels lag markedly behind the normals and cease growth prematurely at 24–30 days.

The cells on the basal surface of the endosperm facing the placental region in normal kernels differentiate into an absorbing tissue at six to 12 days. This differentiation does not occur in the defective caryopsis. Failure to form this absorbing tissue appears from the evidence to be the basic histological manifestation of the action of the *de17* allele.

Many cells in the basal region of the defective endosperm show evidences of collapse at ten days. The breakdown spreads to adjacent cells and to the adjoining central core during the succeeding eight or ten days. The collapse of these cells interrupts the absorption and transfer of nutrients necessary for growth of the seed and contributes directly to the limited development of the latter.

The parenchymatous cells of the placenta are extensively depleted of their total contents by the regularly differentiating normal endosperm. These cells are more slowly and less completely depleted in defective kernels. The difference appears to be a consequence of the unlike absorptive capacities of the normal and defective endosperms.

The somewhat shorter stature of adult *de17* plants, as compared with their normal sibs, may be due either to the handicap incurred at the seedling stage because of poor kernel development or to this factor plus a continuing but only mildly deleterious effect of the *de17* allele on later growth.

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GENETICS OF THE FEMALE PROSTATE IN RATS[1]

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INTRODUCTION

IT IS a well known, but genetically little studied fact, that secondary sex characters have a tendency to become manifest in the contrary sex. Familiar examples are the assumption of nuptial characters, such as cock plumages and changing bill colors by many female birds (WITSCHI 1935, 1936), antlers by the female reindeer, and mammary glands by male mammals. It can hardly be doubted that in all these cases, we deal with a secondary transfer from one to the other sex, rather than persistence of characters of primitive hermaphrodite ancestors.

The situation is more complex when structures pertaining to the genital organs are taken into consideration. In vertebrates, gonochorism is a relatively recent development. Correspondingly, the rudimentary reproductive organs are usually of bisexual character. The embryological separation of males and females occurs relatively late, and involves either the suppression of one of a pair of discrete primordia (e.g., oviducts in males, sperm ducts in females), or the sex-specific secondary differentiation of an originally neutral primordium. This latter course is characteristic for the development of the parts derived from the primitive urogenital sinus. Thus, clitoris and penis both arise from the ventral rim of the cloacal pore. In the evolutionary sense, they are probably of equal age, and only if the clitoris is specifically penis-like in size or shape (as in the extreme case of the spotted hyena, WATSON 1881) can one assume that a partial transfer from the male to the female sex has occurred.

In mammals, *accessory glands of the urogenital sinus region* are usually considered as being characteristic for the male sex. However, bulbo-urethral glands (vestibular glands) attain a considerable size and become secretory in females of several species. Usually, this condition is coincident with the presence of relatively large cavernous bodies and of other parts of the phallic complex. The occurrence of prostatic lobes with active secretory epithelia in adult females, is restricted to fewer species. It is a matter of contention whether some of the urethral glands of the human female actually constitute a prostatic rudiment. RAUTHER (1909) was probably first to observe and describe well-defined female prostates. He found them in the African rat, *Arvicanthis cinereus*, attached to the ventral surface of the vesical urethra. LEHMANN (1938) gave a description of "paravaginal glands" in the primitive insectivore Hemicentetes which probably are dorsolateral lobes of prostate glands. In 1940 BRAMBELL and DAVIS reported the regular presence of well developed ventral prostates in

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females of the African rat, *Mastomys erythroleucus*, obviously a close relative of RAUTHER's species. MATTHEWS (1942) observed well developed female prostates in the East African bat *Coleura afra*. Recently, dorsal prostatic lobes were found in the female cottontail rabbit, *Sylvilagus floridanus* (ELCHLEPP 1946). It is surprising that the sporadic but not infrequent occurrence of ventral prostatic lobes in female rats (*Rattus norvegicus*) was not noticed by any of the early students of rodent anatomy (compare figures 1 and 2). Actually, they were discovered only recently by several investigators engaged in endocrinologic work (MARX 1931; KORENCHEVSKY 1935, 1937; HAMILTON and WOLFE 1937; WITSCHI, MAHONEY, and RILEY 1938; PRICE 1939).

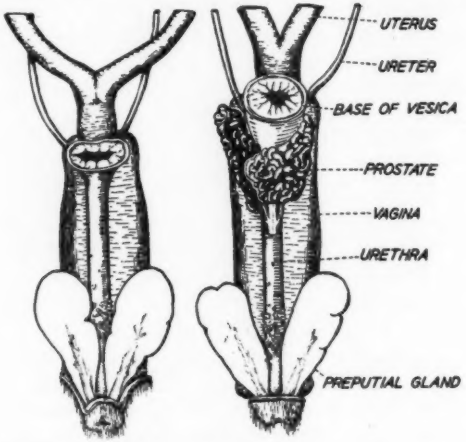

FIGURE 1.—Non-prostate female of the p_9p_9 strain.

FIGURE 2.—Prostate female of the PP strain. Both animals were preserved at the age of 25 days. The lower urethra shows clusters of small urethral glands. A marked swelling of the lower urethra between the preputial glands indicates the location of the clitoris. In figure 2 the ventral wall of the urethra below the prostatic lobes was dissected away to expose the efferent prostatic ducts and their entrance into the urethra.

Camera lucida drawings ×3.

The bulbo-urethral and prostate glands arise from the epithelial wall of the urogenital sinus. In species in which the vagina completely separates from the urethra (as in the murid rodents, some insectivores, bats and lemuroids) these glands, if present, maintain connections with the latter. Consequently, they may be considered as specialized urethral glands (BRUNER and WITSCHI 1946).

Buds of urethral glands sprout from the walls of the urogenital sinus of the female fetuses of most mammals. The wide distribution of such vestiges suggests a considerable antiquity, possibly even an evolutionary derivation from similar glands in hermaphrodite ancestors. However, in the case of the prostatic lobes of female rats, the high degree of specialization as well as the

irregularity of occurrence indicate that we deal with a male sex character that has been transferred to the female.

A study of the inheritance of the female prostate character was undertaken because in the rat the transfer is recent and evidently still progressing. It is hoped that it may contribute toward the understanding of one of the most intriguing phenomena in evolution: the acquisition of useless characters.

LATERALITY

Prostatic lobes appear either bilaterally or unilaterally. In cases of the latter type the right side is highly preferred, the ratio averaging about 10:1. For the sake of simplicity, percentages were calculated only for prostate presence, giving same weight to bilateral and unilateral cases.

ORIGINAL STOCKS

After the discovery of the occurrence of prostates in females of our laboratory stocks, collections of wild gray rats were obtained at the local city dump. Nearly half of a total of 125 females had ventral prostatic lobes (table 1). The origin of this particular population is unknown, and it is impossible to state whether or not this frequency is characteristic for the American rats in general or even for the entire species *Rattus norvegicus*.

TABLE 1

Occurrence of ventral prostatic lobes in 125 gray females collected at the city dump.

	RL	R	L	O	TOTAL
Number	39	14	5	67	125
Percent	31.2	11.2	4.0	53.6	100
		46.4%			

The frequencies of prostates in the females of various stocks recently bred in our laboratory are listed in table 2. A colony of black-hooded rats which was started from a single pair obtained from DR. H. STEENBOCK in 1941 is nearly

TABLE 2

Female prostate occurrence in various unselected laboratory stocks (prior to January 1947).

STOCK	RL	R	L	O	TOTAL	PROSTATE FREQUENCY
CHILD WELFARE hooded	26	17	3	24	70	65.7%
WISTAR albino	55	193	23	668	939	28.9%
LONG ISLAND albino	6	14	3	239	262	8.8%
Black Hooded (p_1p_1)	1	3	—	507	511	0.8%
Cinnamon Hooded	—	—	—	29	29	0.0%

free of the female-prostate character. It was used in some cross-breeding experiments as strain p_1p_1.

The following symbols are used for convenient characterization of selected stocks. *PP* designates a nearly regular occurrence of prostates in females (95–100 percent). On the other hand p_0p_0 indicates the complete absence of female prostates. Between these two extremes p_xp_x stands for any fairly pure breeding stock, x always assuming the value of percent frequency of prostate occurrence. It is one of the objects of this investigation to decide whether the various values from p_0 to P represent a single series of allelomorphs or combinations of multiple factors.

EFFECTS OF SELECTION

It was thought that the differences in female-prostate frequency of unselected groups may arise, in part, as a result of mixture of genetical types. Therefore, separation through selective breeding was attempted.

Prostate Strain PP

The WISTAR rat was used to develop the prostate strain. Females with bilateral prostates were chosen from litters with high prostate occurrence, as

TABLE 3

Effect of selection for high female prostate frequency in the Albino WISTAR *colony.*

PERIOD	RL	R	L	O	TOTAL	FREQUENCY
February '37–December '37	55	193	23	668	939	28%
January '38–July '38	73	88	16	52	229	77%
July '38–October '38	74	52	4	26	156	83%
November '38–March '39	—*	—*	—*	—*	303	93%
April '39–June '40	154	33	5	5	197	97%
June '40–March '42	477	108	8	8	601	99%
April '42–December '46	133	55	7	22	217	90%

* Unknown.

ascertained by laparotomy. They were bred either with their fathers or with their brothers. Later on, the male breeders were chosen on the basis of their progeny records. As may be seen from table 3, the percentage quickly rose above 90, and the ratio of bilateral to unilateral prostates changed from 1:4 to 4:1. Between June 1940 and March 1942, while females without prostates were born only rarely, this strain was used for the first cross-breeding experiment.

The final purification of the prostate strain is unfortunately handicapped by the poor breeder quality of the highly selected males and females. To avoid the risk of losing the entire stock, extreme inbreeding was not attempted.

During the last of the reported periods, selection was no longer exercised and the effect was partly lost (table 3, March 1942 to December 1946). Since the males with the best ancestry records are the poorest breeders, showing almost no sex interest, and the high-bred females care very poorly for their

litters, an unintended negative selection follows the relaxation of rigidly planned breeding.

Low Prostate Strain (p_9p_9)

Attempts at isolation of a genetic strain free of female-prostates from the commercially acquired "LONG ISLAND" stock were not successful. The difficulty of recognizing genetic differences of small magnitude is obvious. When after four years the prostate incidence had resulted in even a slight increase (from 8.8 percent to 9.6 percent) the breeding records were used to segregate "good" and "poor" lines. The best separation permitted the differentiation of genealogic lines with about four and twelve percent frequencies, and their hybrids (compare table 4). A similar breakdown of the records on crossbreeding of

TABLE 4

Tentative segregation of the p_9p_9 colony into two lines, and their hybrids.

PARENTS		FEMALE OFFSPRING					PROSTATE FREQUENCY
MOTHER	FATHER	RL	R	L	O	TOTAL	
(p_4p_4)	(p_4p_4)	2	7	0	207	216	4%
(p_4p_4)	($p_{12}p_{12}$) or ($p_{12}p_4$)	6	17	2	323	348	7%
($p_{12}p_{12}$) or ($p_{12}p_4$)	(p_4p_4)	1	18	0	152	171	11%
($p_{12}p_{12}$)	($p_{12}p_{12}$)	23	60	4	635	722	12%
Total p_9p_9		32	102	6	1,317	1,457	9.6%

p_9p_9 with PP give further support to the assumption of such a mixed character of this strain. Of 86 F_1 females with a p_4p_4 parent, only 46 percent had prostates while of 229 F_1 females with a $p_{12}p_{12}$ or a $p_{12}p_5$ parent, 52 percent showed prostates (total frequency 50.6 percent; compare table 5). In consideration of all facts, it was concluded that this colony did not contain the desired genetic non-prostate type. No further attempt to stabilize p_4p_4 and $p_{12}p_{12}$ strains was made and the entire low prostate strain was discontinued in 1942 when the shortage in technical personnel made a reduction in animal stocks mandatory.

Non-Prostate Strain (p_1p_1)

It was mentioned before, that from a single pair of hooded rats a strain was developed in which female-prostates appear only rarely (4 of a total of 511 cases; table 2). Selection could scarcely be applied here, except that near relatives of the four females with prostates were excluded from the breeding stock. It is doubtful whether this procedure appreciably changes the quality of the strain. The strain is fully fertile in both sexes and is easily propagated.

CROSS BREEDING EXPERIMENTS

a. F_1 Generations

Morphological differences between males of the three genetical strains are

not detectable, but hybrid combinations prove that all transmit the female-prostate character of their strain. The first hybrid generations which are summarized in tables 5 and 6 prove that.

1) female-prostate frequency is transmitted by male as well as female gametes,

2) the female-prostate character is incompletely dominant,

3) with the same prostate strain *PP* the low-prostate (p_9p_9) and non-prostate (p_1p_1) strains give different hybrid frequencies—supporting the view that they represent different genetic types,

4) in reciprocal crosses the offspring of *PP* mothers have a higher female-prostate frequency than the offspring of p_9p_9 or p_1p_1 mothers.

TABLE 5

First generation female offspring from reciprocal crossings of the PP and p_9p_9 strains.

P:	♀ *PP* × ♂ p_9p_9					♀ p_9p_9 × ♂ *PP*					TOTAL F_1 FEMALES
F_1:	RL	R	L	O	TOTAL	RL	R	L	O	TOTAL	
	32	53	2	56	143	33	37	3	100	173	316
	Prostate frequency				61%					42%	50.6%

TABLE 6

First generation female offspring from reciprocal crossings of the PP and p_1p_1 strains.

P:	♀ *PP* × ♂ p_1p_1					♀ p_1p_1 × ♂ *PP*					TOTAL F_1 FEMALES
F_1:	RL	R	L	O	TOTAL	RL	R	L	O	TOTAL	
	4	16	1	14	35	2	8	1	27	38	73
	Prostate frequency				60.0%					28.9%	43.9%

The fourth point calls for further comment. The difference between the reciprocal groups is statistically significant, in both combinations (table 5 and table 6). In the first one it can be shown that the high frequency among the Pp_9 females is partly the result of the mixed nature of the p_9p_9 parent stock. But this cannot explain the entire difference. Sex-linked inheritance is ruled out since all females carry a paternal as well as a maternal X chromosome. There remain two possible explanations for consideration. During fetal development some physiological factor might shift manifestations of the character toward the maternal type, or the males of the *PP* strain might not have been as uniformly and not as highly selected as the females. No direct support for the former alternative was found. On the other hand it is at least conceivable that the genetic purification of the males becomes delayed, because they do not directly exhibit the genic character and selection is made only on the basis of breeding records. In other words, it is possible that the males were not all of a *PP* constitution but partly hybrids, for example, of Pp_{80} or similar type. For reasons explained above, such hybrids may unintentionally have

been given preference in setting up mating combinations. The question will be discussed again in connection with the results of backcrosses.

b. Back-Cross Generations

If, as implicitly assumed so far, the prostate factor is monogenic, backcrossing F_1 with the recessive parental stock must yield equal numbers of hybrid and of homozygous recessive females.

Low-prostate hybrids × low-prostate stock (table 7)

The prostate incidence in the hybrid group was 50.6 percent (table 5), in the p_9p_9 stock 9.6 percent (table 4). In the backcross the expected frequency is therefore 30.1 percent; with this the observed 28.4 percent (table 7) compares favorably.

TABLE 7

Backcrosses of F_1 females and males with the low-prostate strain.

	PARENTS		FEMALE OFFSPRING	
	FEMALE	MALE	NUMBER	PROSTATE FREQUENCY
1)	Pp_9	p_9p_9	320	30%
2)	p_9P	p_9p_9	428	24%
3)	p_9p_9	Pp_9	52	29%
4)	p_9p_9	p_9P	305	32%
	Total		1,105	28.4%

Non-prostate hybrids × non-prostate stock (table 8)

The prostate frequency was 43.9 percent in hybrids (table 6) and 0.8 percent in the p_1p_1 stock (table 2). Therefore, the calculated expectancy for the backcross generation is 22.4 percent. Since the average of this experiment is based on relatively small numbers, the observed 15 percent incidence (table 8) is a satisfactory approximation.

If the original strains are homozygous and the F_1 generation is genetically uniform, the F_1 females *without* prostates should transmit the same frequency as F_1 females *with* prostates. This expectation is tested by a breakdown of combination 1 of table 7. It shows that 20 females *with* prostates littered 176 B.C. females of which 29 percent had prostates, while 13 females *without* prostates produced 144 B.C. females, 31 percent with prostates. Evidently the manifestation of the prostate character is independent of actual presence or absence of prostates in the mother. Therefore, it appears that manifestation (in F_1) does not depend on a supporting action of modifying genes.

The analogous analysis of combination 2 (table 7) gives a somewhat different picture. From 17 females *with* prostates, 134 B.C. females were obtained with a prostate occurrence of 33 percent; but 29 females *without* prostates produced 294 daughters of which only 19 percent had prostates. This low frequency indicates that some of the 29 F_1 females without prostates carried a paternal prostate factor which was not P but one of lower value, possibly p_9,

or less. Therefore, the analysis of the backcross data corroborates the assumption that some males of the prostate strain were not of the highest class obtainable by selection. What appeared as a shift in the maternal direction in the F_1 generation is now shown to have a genetic basis. It is the consequence of a residual impurity of the selected male stock.

c. F_2 Generations

The four possible second offspring combinations of the $PP \times p_1p_1$ crossings were obtained. The total number of F_2 females is too low to give this experiment much weight, though it is gratifying that the outcome is in good agreement with the calculated expectation of prostate frequency (table 9).

TABLE 8

Backcrosses of F_1 females and males with the nonprostate strain.

	PARENTS		FEMALE OFFSPRING	
	FEMALE	MALE	NUMBER	PROSTATE FREQUENCY
1)	Pp_1	p_1p_1	49	16%
2)	p_1P	p_1p_1	47	13%
3)	p_1p_1	Pp_1	3	13%
4)	p_1p_1	p_1P	5	
	Total B.C.		104	15%

TABLE 9

Second generation female offspring of $PP \times p_1p_1$ crossings.

F_1 COMBINATIONS	F_2 FEMALE OFFSPRING				TOTAL
	RL	R	L	O	
$Pp_1 \times Pp_1$	2	2	—	7	11
$Pp_1 \times p_1P$	6	1	1	4	12
$p_1P \times Pp_1$	2	3	—	4	9
$p_1P \times p_1P$	5	8	—	13	26
Total	15	14	1	28	58
Frequency observed		52%			
Frequency expected		47%			

DISCUSSION

In amphibians, the development of secondary sexual differences is entirely hormone controlled. Transplantation of gonads from one sex to the other, or experimental reversal of the sex of the gonads is followed by a complete change of all somatic sex characters. Technically, we may speak of *hormone-controlled manifestation*. In the amniote vertebrates a new type of control has developed

which permits sexual differentiation of auxiliary sex characters to develop at early prepuberal stages, before sex hormones are produced in appreciable quantities. It is clearly linked with the sex determining chromosome mechanism, but does not follow the so-called sex-linked mode of inheritance. Like primary sex differentiation, it depends on the balance between the X and Z chromosome complexes, and it is therefore proposed to call this an *X/Z controlled manifestation.** Both types of control usually result in a sex-limited development of secondary characters, though notable exceptions to this rule are known to occur. Thus in the starling, the female produces considerable amounts of an androgenic hormone, and causes some male secondary characters to develop during every breeding season (WITSCHI and MILLER 1938). Since chromosome control, like the better known hormone control, must be essentially a chemical process, the spontaneous formation of prostate glands in female rats of the *PP* strain may originate in an analogous way, that is, from a changed X/Z ratio. In other words, prostatism may indicate a shift of the genetic balance of the female toward a hermaphrodite constitution. The previously mentioned observations on the changed reproductive behavior and competence of the *PP* strain would find an immediate explanation on the principle of such an interpretation. Since the rat has sex chromosomes of the XY type and the factor responsible for female prostate development is not sex-linked, it follows that the mutated gene should be located in the Z chromosome and increase its male determining potency.

On the other hand, there remains the possibility that the balance of sex determining genes is unaltered, and the change has occurred in a more specific gene—one which affects the responsiveness of the prostatic region of the fetal urogenital sinus. The concurrent physiological modifications would have to find a separate explanation.

No decision on these alternative interpretations can be reached on the basis of the presented material here. A more exhaustive discussion would have to include a vast body of comparative data, and carry beyond the scope of the present contribution. A complete discussion would also have to expand on the fact that early hormone treatments considerably increase the area of prostate production in female rodents. The bearing of these experiments on the theory of gene action and morphogenic induction has been pointed out in earlier publications (MAHONEY 1942; BRUNER and WITSCHI 1946).

SUMMARY AND CONCLUSIONS

Hereditary prostate formation in female rats may reach a penetrance of 99 and possibly 100 percent.

* One has often spoken of a balance between sex chromosomes and autosomes; but among the "autosomes" there must be at least one pair of chromosomes that are mainly or entirely responsible for the "autosomal" component. While the XX (or XY) chromosomes carry mostly or exclusively female determining factors, the male determining genes are located in a second pair, the ZZ (or ZW) chromosomes. Either one of the two pairs of sex chromosomes may serve as a basis for the evolution of a heterochromosome mechanism of sex inheritance. In the rat this role fell to the XX pair.

The determining factor is monogenic and occurs in a series of quantitative alleles.

It is transmitted identically by both sexes (not sex-linked).

In hybrids of high and low prostate strains, manifestation of the character is intermediate.

Groups with low manifestations, whether of pure bred or of hybrid stock, show a prevalence of unilateral, mostly dextral prostate cases.

Hybrid females from carefully selected stocks breed the same whether they have prostates or not. Manifestation apparently is not influenced by modifying genes.

Like primary sex differentiation, the sex-limited occurrence of prostates in males depends on the ratio between the X and Z chromosome complexes (X/Z controlled manifestation). The appearance of prostates in females possibly is caused by a shift in the genic balance of the female toward the hermaphrodite type; though alternative interpretations are not excluded.

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METHODS OF COMBINING LINKAGE INTENSITY VALUES FROM BACKCROSS, F_2 AND F_3 GENETIC DATA[1]

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SEVERAL sources of genetic data for the calculation of linkage intensities (percentages of recombination) may be available including F_2 and F_3 data from both coupling and repulsion crosses. For the various sources of data involving two pairs of factors each segregating 3:1 in F_2 with all classes fully viable, IMMER (1934) has presented maximum likelihood and standard error formulae. IMMER and HENDERSON (1943) have presented tables which greatly reduce the necessary calculations. Recently, FISHER (1946) has presented a simple method of scoring linkage data based on maximum likelihood formulae which can be used to obtain (1) a test of significance of the deviations from independent assortment for each source of data as well as for the pooled data, (2) a test of the homogeneity of the group of linkage intensities to be combined, and (3) the average or combined p value. Two methods have been suggested for obtaining a single recombination value which best fits all the data: (1) by combining (by addition) the separate maximum likelihood formulae into one equation and solving by trial and error substitution of various values for p until one is found which most nearly makes the equation equal to 0 (IMMER, 1934); and (2) approximating the combined p value by weighting each separate recombination value by appropriate use of the amount of information furnished by each source of data (IMMER and HENDERSON, 1943). In this latter publication only the second method was used.

In trials of the first method in a problem used in advanced classes at the UNIVERSITY OF MINNESOTA, it was found that certain of the maximum likelihood formulae as they now exist in the literature must be modified to accomplish the desired purpose. The purpose of this paper is to point out these changes, to illustrate the use of the formulae in determining p values and standard errors, to adapt and illustrate FISHER's method of scoring, and to summarize the simplest methods available. To enable workers to derive their own formulae for linkage data involving other genetic ratios, the steps in deriving them are given in some detail.

DERIVATION OF THE FORMULAE

If the percentage of recombination is the same in male and female, and p represents the recombination value in repulsion expressed as a decimal, $1-p$

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being the frequency of parental combinations, the expected frequencies of F_1 gametes and F_2 phenotypes from a *repulsion* cross involving factor pairs Aa and Bb are:

F_1 gametes or F_2 phenotypes	*AB*	*Ab*	*aB*	*ab*	Total
F_1 gametic frequency	p/2	(1−p)/2	(1−p)/2	p/2	1
F_2 expected phenotypic frequency	$(2+p^2)/4$	$(1-p^2)/4$	$(1-p^2)/4$	$p^2/4$	1
F_2 observed number	a	b	c	d	n

The expected frequencies of the F_2 genotypes and their observed numbers (d to m), as determined by their breeding behavior in F_3 lines are in the cells of the following checkerboard table:

	AA	*Aa*	*aa*
BB	$p^2/4$ (e)	$2p(1-p)/4$ (f)	$(1-p)^2/4$ (l)
Bb	$2p(1-p)/4$ (g)	$2p^2/4$ (h) $2(1-p)^2/4$ (i)	$2p(1-p)/4$ (m)
bb	$(1-p)^2/4$ (j)	$2p(1-p)/4$ (k)	$p^2/4$ (d)

The observed numbers, h and i, are for the two kinds of heterozygotes, coupling (AB/ab) and repulsion (Ab/aB) respectively.

For repulsion, the steps in the derivation of any maximum likelihood formula may be illustrated for the singly dominant Ab F_2 phenotype (j and k classes) classified in F_3[2] as follows:

1. The total of the expected frequencies of the component genotypes AAbb and Aabb is determined:

$$(1 - p)^2/4 + 2p(1 - p)/4 = (1 - p^2)/4.$$

2. The expected proportion for each class is obtained, expressed in its simplest terms:

$$\text{AAbb} = \frac{(1 - p)^2/4}{(1 - p^2)/4} = \frac{(1 - p)}{(1 + p)}$$

$$\text{Aabb} = \frac{2p(1 - p)/4}{(1 - p^2)/4} = \frac{2p}{(1 + p)}.$$

3. The observed number in each class is multiplied by the logarithm of the expected frequency in that class and summed:

$$j \log [(1 - p)/(1 + p)] + k \log [2p/(1 + p)]$$

which may be written:

$$j \log (1 - p) - j \log (1 + p) + k \log 2p - k \log (1 + p). \qquad (1)$$

4. Differentiate formula 1 above with respect to p, simplify, and equate to 0:

[2] This one is chosen because the total is not 1. The total for the F_2 phenotypes is 1 making it a poor case for illustration.

$$\frac{k}{p} - \frac{j}{(1-p)} - \frac{(j+k)}{(1+p)} = 0. \tag{2}$$

This is the maximum likelihood formula. It may be expressed in the following form for direct calculation of p:

$$p = \frac{k}{k+2j}. \tag{3}$$

For coupling, the maximum likelihood equation may be derived most easily by substituting $(1-p)$ for p in formula (1) above. Differentiating with respect to p, simplifying and equating to 0 gives:

$$-\frac{k}{(1-p)} + \frac{j}{p} + \frac{(j+k)}{(2-p)} = 0. \tag{4}$$

A comparison of formulae (2) and (4) shows that the coupling formula may be obtained from the one for repulsion by substituting $(1-p)$ for p and multiplying the entire equation by (-1).[3] This also holds for the other formulae when used for coupling data. This change of sign for coupling is necessary when repulsion and coupling formulae are combined to obtain a p value which best fits all of the available data. It is necessary also when FISHER's method of scoring linkage data is used, as he has indicated.

Another source of difficulty arises from the fact that in certain formulae (IMMER 1934, Formula I), a factor common to each term has been dropped. This factor, since it is not common to all formulae, must be retained when data from several sources are combined. This factor may be illustrated by the derivation of the maximum likelihood equation for F_2 data in repulsion as follows:

The equation to be maximized by differentiation with respect to p is:

$$a \log (2+p^2)/4 + b \log (1-p^2)/4 + c \log (1-p^2)/4 + d \log p^2/4.$$

By differentiation and simplification, this becomes

$$2p\left[\frac{a}{(2+p^2)} - \frac{b+c}{(1-p^2)} + \frac{d}{p^2}\right] = 0. \tag{5}$$

For determining a p value from this source of data only, the factor $2p$ may be dropped and the simpler equation $-np^4+(a-2b-2c+d)p^2+2d=0$ used. This, being in the form of the quadratic equation $mx^2+nx+r=0$, is solved for p^2 by the general formula $x=\frac{-n\pm\sqrt{n^2-4mr}}{2m}$. If the proper signs

[3] IMMER and HENDERSON (1943), not recognizing the necessity for this change of sign, resorted to the method of weighting to combine repulsion and coupling data. Later, IMMER discovered this as a source of difficulty and the senior writer proved that the change was necessary by differentiating the appropriate coupling formulae. FISHER (1946) uses the change of sign but derived his formulae for coupling instead of repulsion data.

TABLE 1

Summaries of maximum likelihood formulae for determining p *values from different sources of data involving two factor pairs; including formulae for the direct calculation of* p *and the amount of information.*

SOURCE OF DATA	UNREDUCED FORMULAE*	FORMULAE FOR DIRECT DETERMINATION OF p†	AMOUNT OF INFORMATION (i) PER‡ INDIVIDUAL
Repulsion:			
Backcross	$\frac{(a+d)}{p}-\frac{(b+c)}{(1-p)}=0$	$p=\frac{(a+d)}{(a+b+c+d)}$	$\frac{1}{p(1-p)}$
F_2	$+2p\left[\frac{a}{(2+p^2)}-\frac{(b+c)}{(1-p^2)}+\frac{d}{p^2}\right]=0$	$-np^4+(a-2b-2c-d)p^2+2d=0$	$\frac{2(1+2p^2)}{(1-p^2)(2+p^2)}$
F_2 from singly dominant F_2's (Ab)	$+\frac{k}{p}-\frac{j}{(1-p)}-\frac{(j+k)}{(1+p)}=0$	$p=\frac{k}{(k+2j)}$	$\frac{2}{p(1+p)(1-p^2)}$
F_2 from singly dominant F_2's (aB)	$+\frac{m}{p}-\frac{l}{(1-p)}-\frac{(m+l)}{(1+p)}=0$	$p=\frac{m}{(m+2l)}$	$\frac{2}{p(1+p)(1-p^2)}$
F_2 from doubly dominant F_2's (AB)	$+\frac{(2e+f+g)}{p}-\frac{(f+g)}{(1-p)}-\frac{2(h+1)(1-2p)}{(1-2p+2p^2)}-\frac{2p(e+f+g+h+i)}{(2+p^2)}=0$		$\frac{4(2-6p+3p^2+4p^3)}{p(1-p)(2+p^2)^2(1-2p+2p^2)}$
F_2 separation of doubly het. F_2 plants into AB/ab and Ab/aB types	$+\frac{2h}{p}-\frac{2i}{(1-p)}+\frac{2(h+i)(1-2p)}{(1-2p+2p^2)}=0$		$\frac{4}{(1-2p+2p^2)^2}$

* In coupling, substitute $(1-p)$ for p and multiply each entire formula by (-1).

† In coupling, solution of these gives values for $(1-p)$.

‡ In coupling, substitute $(1-p)$ for p.

are observed, the square root of the positive solution for x will give p (since $x=p^2$). When combined with other formulae into a single maximum likelihood equation this factor $2p$ must be retained and the unreduced formula (5) used. The unreduced formulae to be used for the various types of linkage data in repulsion are summarized in table 1. Formulae for the calculation of p values directly, as well as the amount of information per individual, are included in this table. The necessary changes for their use with coupling data are also indicated.

DERIVATION OF STANDARD ERROR FORMULAE

In developing formulae for the standard error of p, FISHER took the second differential of the maximum likelihood expression, and by substituting the expected for the observed values a, b, c, d, etc., obtained an expression giving the amount of information (i) furnished per individual. MATHER (1938, pages 57, 59) has given a general formula for deriving i:

$$i = S\left(\frac{1}{m}\left(\frac{dm}{dp}\right)^2\right) \tag{6}$$

where m is the expected proportion of the total in each class, dm/dp is the derivative of m with respect to p, and S indicates summation. This formula may be valuable in planning experiments, since it is possible to determine the amount of information furnished by each genotypic class. Using again the singly dominant F_2 Ab phenotype classified in F_3 for illustration, the calculations may be tabulated as follows:

CLASS	m	$\frac{dm}{dp}$	$\left(\frac{dm}{dp}\right)^2 \times \frac{1}{m}$	i
j	$\frac{1-p}{1+p}$	$\frac{-2}{(1+p)^2}$	$\frac{4}{(1+p)^4} \times \frac{1+p}{1-p}$	$\frac{4}{(1+p)^3(1-p)}$
k	$\frac{2p}{1+p}$	$\frac{2}{(1-p)^2}$	$\frac{4}{(1+p)^4} \times \frac{1+p}{2p}$	$\frac{2}{(1+p)^3p}$

The sum of the last column reduces to $i=\frac{2}{p(1+p)(1-p^2)}$.

Substituting a value of p in this formula gives, for that amount of recombination, the average amount of information per singly dominant F_2 individual classified in F_3.

When multiplied by n, the number of F_2 individuals thus classified in F_3 lines, the total amount of information (I) furnished by these data is given:

$$I = ni = \frac{2n}{p(1+p)(1-p^2)}.$$

Since as MATHER states it, "the greater the amount of information concerning the recombination fraction, the greater the precision, or the less the variance, of the estimate . . . ," the variance of p has been defined as the reciprocal of the total information (I). Therefore

$$SE_p = \sqrt{\frac{1}{I}} = \sqrt{\frac{p(1+p)(1-p^2)}{2n}}.$$

The formulae for the amount of information per plant or line in each source of data are in table 1.

For an exact standard error of the combined p value which best fits all the data, calculate the total amount of information for each source of data, based in each case on the combined p value. The standard error is then: $\sqrt{1/SI}$. This standard error is approximated by the same formula in which the I for each source of data is based on its own respective p value.

APPLICATION OF THE FORMULAE

The application of the formulae may be illustrated by using data of ROBERTSON *et al.* (1944) in barley. The factors involved were *Vv*, non six row versus six row; and *Ff*, green versus chlorina plant color. The repulsion cross was *Triple-Bearded Mariout*×*Minnesota* 84-7 and the coupling cross was *Englawnless* ×*Minnesota* 84-7-6. The data are summarized in table 2. The F_2 doubly heterozygous plants were not separated into *AB/ab* and *Ab/aB* types. If such data were available, they would be incorporated as an additional source and treated as illustrated for the others. However, as pointed out by IMMER (1934), this separation is seldom worthwhile for recombination percentages exceeding 22.

TABLE 2

Linkage data in F_2 and F_3 from a repulsion and a coupling cross involving v *and* f *in barley.* (*From* ROBERTSON et al., *1944.*)

	REPULSION				COUPLING			
F_2	a=753	b=292	c=351	d=19	a=1064	b=223	c=259	d=218
F_3		*VV*	*Vv*	*vv*		*VV*	*Vv*	*vv*
	FF	e=13	f= 85	l=121	*FF*	e=139	f=105	l=30
	Ff	g=102	h+i=278	m=93	*Ff*	g=109	h+i=316	m=115
	ff	j=109	k=81		*ff*	j=19	k=113	

A p value was first calculated for each source of data by substituting the observed numbers in table 2 for a, b, c, d, etc. in the formulae in table 1. For example, for singly dominant *Ab* F_2 plants from the coupling cross classified in F_3 the unreduced maximum likelihood formula (observing the change of signs for coupling) is:

$$-\frac{113}{p}+\frac{19}{(1-p)}+\frac{132}{(1+p)}=0.$$

Solving by trial and error substitution of values of $1-p$ for p in this formula gave p=.748 for which the recombination value is (1−.748)=.252 or 25.2 percent. The formula for direct calculation of p gives the same result. The

amount of information per F_3 line is $\frac{2}{.748(1+.748)(1-.748^2)}=3.4725$.

Values of p and the amount of information per F_2 plant or F_3 line for each source of data are summarized in columns 2 and 3 of table 3. The standard errors are in column 6, and the figures from which they are calculated are in columns 3 to 5.

TABLE 3

Values of p calculated by the method of maximum likelihood from the data in table 2, together with standard errors, the figures necessary for S.E. calculation, and for weighting p *values.*

SOURCE OF DATA	p VALUE	i	n	I=ni	S.E.=$\sqrt{1/\mathrm{I}}$	pI
Repulsion						
F_2	.244	1.1555	1,415	1,635.0	.0247	398.94
$F_3(Ab)$	.271	6.2667	190	1,190.7	.0290	322.68
$F_3(aB)$	.278	6.1008	214	1,305.6	.0277	362.96
$F_3(AB)$	.265	5.4563	478	2,608.1	.0196	691.15
Coupling						
F_2	.318	2.9279	1,764	5,164.8	.0139	1,642.41
$F_3(Ab)$	.252	3.4725	132	458.4	.0467	115.52
$F_3(aB)$	.343	3.2324	145	468.7	.0462	160.76
$F_3(AB)$	.279	3.8393	669	2,568.5	.0197	716.61

It would be desirable to have one recombination value which best fits all these data. The method of approximating such a combined recombination value by weighting the separate p values will be illustrated first. The formula is:

$$\text{combined p value by weighting} = \frac{\mathrm{SpI}}{\mathrm{SI}} \qquad (7)$$

where S, p and I are summation, p value, and information respectively. Each value of p is weighted by multiplying it by the total amount of information (I) furnished by that source of data, as shown in the last column of table 3. The standard error for this average p value is $\sqrt{1/\mathrm{SI}}$. For example, to combine F_2 repulsion data and F_3 coupling data from singly dominant F_2 *Ab* plants the average p value is $\frac{398.94+115.52}{1,635.0+458.4}=.246$ and the approximate standard error for this average p value is $\sqrt{\frac{1}{1,635.0+458.4}}=.0219$. Average p values for data combined in various ways by weighting, together with their approximate standard errors, are listed in table 4.

The second method of obtaining a p value which best fits all the data is to combine the separate maximum likelihood expressions into one equation and

TABLE 4

Average p values for various combinations of the separate values in table 3 as determined by weighting, by combining maximum likelihood formulae, and by scoring.

SOURCE OF DATA	AVE. p BY WEIGHTING	AVE. p BY MAXIMUM LIKELIHOOD	AVE. p ESTIMATED FROM SCORES AT $p=.5$	AVE. p ESTIMATED FROM SCORES AT $p=.285$
Repulsion				
F_2 Singly Dominant ($Ab+aB$)	27.5±2.00	27.4±2.00	23.4	27.4
All F_2	27.0±1.40	26.9±1.40	25.3	26.9
F_2 and F_3	26.3±1.22	26.4±1.20	29.0	26.4
Coupling				
F_2 Singly Dominant ($Ab+aB$)	29.8±3.28	30.1±3.31	32.4	30.1
All F_2	28.4±1.69	28.5±1.71	28.8	28.5
F_2 and F_3	30.4±1.07	30.6±1.09	28.1	30.6
Repulsion and Coupling	28.6±0.81	28.9±0.81	28.5	28.9

then solve for p by trial and error substitution. This may be illustrated for the two sources of data used above, the calculations being as follows:

SOURCE OF DATA	FORMULA	FIRST TRIAL		SECOND TRIAL	
		p VALUE	REMAINDER	p VALUE	REMAINDER
F_2 repulsion	$+2p\left[\frac{753}{(2+p^2)}-\frac{643}{(1-p^2)}+\frac{19}{p^2}\right]$	.246	−2.46	.2461	−2.61
F_2 coupling from Ab F_2's	$-\frac{113}{p}+\frac{19}{(1-p)}+\frac{132}{(1+p)}$	.754	+2.63	.7539	+2.57
Sum			+.17		−.04

The net positive remainder of +.17 obtained when the recombination value of .246 was substituted into the formulae indicates that the true p value is slightly higher. It is however slightly lower than .2461. If the signs as indicated in table 1 are used, a positive remainder will always indicate that the true p value is higher than the one tried and a negative one will indicate a lower p value than the one tried. A method of determining the correction to be used is presented in connection with FISHER's method of scoring presented later. The exact standard error is $\sqrt{1/I}$ where I is determined by substituting this combined p value in each of the formulae concerned (last column of table 1). In this case,

$$I = \frac{2 \times 1{,}415[1 + (2 \times .246^2)]}{(1 - .246^2)(2 + .246^2)} - \frac{2 \times 132}{(.754)(1 + .754)(1 - .754^2)}.$$

Various average p values obtained by this method, together with their exact standard errors may be compared with those obtained by weighting (with approximate standard errors), in table 4. From this table it is apparent that the values obtained by weighting approximate closely the true value obtained by maximum likelihood. They also involve appreciably less calculation.

THE METHOD OF SCORING

The method of scoring explained by FISHER (1946) will now be adapted and illustrated. If, in the unreduced expressions in table 1 (not equated to

TABLE 5

Formulae for scores of maximum likelihood formulae and for amount of information when $p=.5$.

SOURCE OF DATA	FORMULAE* FOR SCORES (c) AT $p=.5$	INFORMATION PER F_2 PLANT OR F_3 LINE (i) AT $p=.5$
Backcross	$2(a-b-c+d)$	4
F_2	$4\left(\frac{a}{9}-\frac{b+c}{3}+\frac{d}{1}\right)$	16/9
F_3 from Ab F_2 plants	$\frac{4(k-2j)}{3}$	32/9
F_3 from aB F_2 plants	$\frac{4(m-2l)}{3}$	32/9
F_3 from AB F_2 plants	$\frac{4(8e-f-g-h-i)}{9}$	128/81
F_3 from doubly heterozygous F_2 plants	$4(h-i)$	16

* Signs appropriate for repulsion, reverse for coupling.

zero), we let $p=0.5$, the value for independent assortment, and substitute this value (1/2) for p in the formulae, the simple expressions given in table 5 are obtained. Now, by substituting the observed values in table 2 for a, b, c, etc. in these expressions, a remainder or score (c) for each source of data is obtained at 50 percent recombination. The last column of table 5 is the amount of information per F_2 plant or F_3 line and is obtained by substituting $p=1/2$ in the formulae in the last column of table 1. These values need only be multiplied by n, the number of plants classified from each source to obtain the total amount of information (I) furnished by that source at 50 percent recombination. Since FISHER has shown that c^2/I is distributed as χ^2, a test of the significance of the deviation from 50 percent recombination is available for each source of data, each χ^2 value having one degree of freedom. Similarly $\chi^2=(Sc)^2/SI$ will test the deviation from 50 percent for the pooled data with one degree of freedom. The difference $\chi^2=S\left[\frac{c^2}{I}\right]-\frac{(Sc)^2}{SI}$ will test heterogeneity

and will have (N−1) D.F. where N is the number of sources of data pooled. These calculations are summarized in the first half of table 6. The negative signs of the scores indicate less than 50 percent recombination. The individual as well as the total χ^2 values at $p=.5$ show, without exception, a significant deviation.

TABLE 6

Scores and total amount of information at $p=.5$ and $p=.285$, χ^2 tests of significance (goodness of fit to assumed p value, and homogeneity), and calculations to obtain estimates of the true combined p value, utilizing the data in table 2.

SOURCE OF DATA	$p=.5$ SCORE (c)	$p=.5$ I	$p=.5$ $\chi^2=c^2/I$	$p=.285$ SCORE (c)	$p=.285$ I	$p=.285$ $\chi^2=c^2/I$
Repulsion:						
F_2	−446.66	2,515.6	79.31	− 59.34	1,720.4	2.04
$F_3(Ab)$	−182.67	675.6	49.39	− 16.10	1,129.3	.23
$F_3(aB)$	−198.67	760.9	51.87	− 9.45	1,272.0	.07
$F_3(AB)$	−160.44	755.3	34.08	− 48.62	2,290.0	1.03
Coupling:						
F_2	−702.22	3,136.0	157.24	+194.47	5,813.2	6.51
$F_3(Ab)$	−100.00	469.3	21.31	− 14.41	440.5	.47
$F_3(aB)$	− 73.33	516.6	10.43	− 28.98	483.9	1.74
$F_3(AB)$	−258.67	1,057.2	63.29	− 14.97	2,480.7	.09
Sum	−2,122.66	9,885.5	466.92	+ 60.56	15,630.0	12.18
Total χ^2=	$-2,122.66^2 \div 9,885.5$	=	455.79	$60.562 \div 15,630.0$	=	.23
Heterogeneity χ^2			11.13			11.95
Correction=	$-2,122.66 \div 9,885.5 = -.2147$			$+60.56 \div 15,630.0$	=	+.0039
	Estimate of correct $p=.5-.2147=.2853$			Estimate of correct $p=.285+.0039=.2889$		

The heterogeneity χ^2 value of 11.13 for 7 D.F. shows that the data may be considered homogeneous and hence suitable for combining. FISHER has shown that the ratio Sc/SI will provide an efficient estimate of the degree of deviation and therefore give a basis for estimating p. In the example presented this ratio was −.2147 and subtracting from .5 gives a provisional estimate of p at 28.53 percent recombination.

The formulae in table 1 may now be solved for $p=.285$. The scores obtained by this substitution and the total information for each source are in the last half of table 6. With the exception of the F_2 coupling data which by the positive score indicates a recombination value greater than .285, no significant deviation from $p=.285$ is indicated and the heterogeneity χ^2 remains essentially the same. FISHER suggests using a value of p fairly close to the actual when testing for heterogeneity. The second estimate of p is .289 which agrees with that obtained by trial and error substitution of the combined repulsion and coupling formulae. Estimates of p for certain combinations of data based on

scores for $p = .5$ and $p = .285$ are given in table 4. In all cases when a score based on $p = .285$ was used, the correct value of p was obtained even though the correction in some cases exceeded two percent.

DISCUSSION

It may be well, at this point, to summarize the simplest methods of determining p values, their standard errors, and the information per plant or line for backcross, F_2 and F_3 data for (3:1)(3:1) segregations with complete dominance, no factor interaction, and all classes fully viable.

1. For backcross data, use direct calculation of percent recombination in the total population. For the standard error, use "factors to be divided by $\sqrt{n}$," in the last column of table 1 of IMMER and HENDERSON (1943). Amount of information per plant (i) is the reciprocal of this factor squared ($i = 1/f^2$).

2. For F_2 coupling or repulsion data alone, use the product method. The p values may be determined directly from the ratios ad/bc for repulsion and bc/ad for coupling in table 1 of IMMER and HENDERSON (1943). For standard errors, use "factors to be divided by $\sqrt{n}$" in the same table. Amount of information per plant is again the reciprocal of this factor squared. STEVENS (1939) has presented tables for p values determined to three places by the product method and gives a table of values, which, to obtain the standard error, must be divided by n followed by extraction of the square root.

3. For F_3 data from the singly dominant F_2 phenotype, p values may be determined from table 3 of IMMER and HENDERSON (1943) carrying the ratios of number of heterozygous/number of homozygous F_3 lines corresponding to different p values. In practice, such data for Ab and aB F_2 phenotypes may be combined. In the same table are "factors to be divided by $\sqrt{n}$" to obtain the standard error; also the amount of information per F_3 line.

4. For F_3 data from doubly dominant F_2 phenotypes, the maximum likelihood formula must be solved for p by trial and error substitution. IMMER and HENDERSON (1943) in table 5 give "factors to be divided by $\sqrt{n}$" to obtain the standard error; also the amount of information per F_3 line.

5. For F_3 data from doubly heterozygous F_2 plants classified into coupling and repulsion types, the value for p, the amount of information per F_3 line, and the standard error must be calculated from the maximum likelihood formulae.

6. For a combined p value which best fits all available data, the method of scoring explained by FISHER (1946) and illustrated here for the genetic ratios under consideration appears to offer the easiest solution. Data may be scored first for 50 percent recombination from the formulae presented in table 5. The estimated value of p obtained from these scores may be used for solution of the unreduced maximum likelihood formulae summarized in table 1 to obtain a second series of scores from which the average p value is obtained. In case p values for the different sources of data have been calculated, a close approximation to the true combined p value may be obtained by the method of weighting, formula (7) in this paper. After an average p value is obtained,

the calculation of the standard error will be facilitated by reference to the appropriate tables referred to above.

For ratios other than (3:1)(3:1) IMMER (1930) has presented formulae for obtaining p values based on the product method, and their *probable errors* and has presented tables to facilitate their calculation.

SUMMARY

When maximum likelihood formulae for different sources of linkage data are combined into one equation whose solution of p gives the best fit to all available data, the unreduced formulae, as presented in table 1 must be used. The separate formulae, as listed for calculating p values from different sources of data, are for repulsion. When these formulae are used for coupling data, $(1-p)$ must be substituted for p and the entire formula multiplied by (-1). When data from different sources are not being combined, simplified formulae, also in table 1, may be used for the direct calculation of p or appropriate tables to facilitate the calculations may be used as indicated in the discussion.

The general methods of derivation of maximum likelihood and of standard error formulae are given, their use is illustrated, and a discussion of the simplest procedures which may be used both in calculating separate p values and a combined p value for all data available is included.

The use of FISHER's method of scoring in determining: significance of deviations from independence, the homogeneity of the linkage values, and a combined p value for all sources of data is also illustrated.

Only the case involving linkage between two pairs of factors, each of which segregates in a 3:1 ratio in F_2 is considered.

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MITOTIC NONDISJUNCTION IN THE CASE OF INTERCHANGES INVOLVING THE B-TYPE CHROMOSOME IN MAIZE[1]

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INTERCHANGES between the B-type chromosome and members of the basic set (A-type chromosomes) in maize provide a means of clarifying the anomalous behavior of the B-type itself. They also make possible a method whereby plants deficient for a specific A-type segment or carrying the segment as a duplication may be regularly produced for use in various cytogenetic studies.

The characteristics of the B-type have been described in detail by RANDOLPH (1941). The chromosome occurs as a supernumerary in many strains of maize. Within a strain, the number of B-types may vary considerably from plant to plant. RANDOLPH (1928) found a range of zero to eight among 43 plants of the Black Mexican variety. The chromosome does not produce a specific genetic effect even when present in relatively large numbers; a plant devoid of B-types is not noticeably different in appearance from one carrying as many as ten (RANDOLPH 1941).

The source of the variability in B chromosome number within a strain has been investigated in crosses in which only one of the parents furnished the B-types. The results of two such crosses and their reciprocals are given in table 1. On an orthodox basis, the progeny from these crosses should not have more than one B chromosome. Each of the crosses, however, yielded plants with two or more B-types and the proportion of these exceptional plants was particularly high when the B-types were transmitted by the male parent (i.e., in the oB×1B and oB×2B crosses).

These results are due only in small part, if at all, to aberrant meiotic behavior. In the microsporocytes of a 2B plant, for example, the B chromosomes usually form a bivalent in the first meiotic prophase and disjunction follows. The regularity of this procedure may be verified by chromosomal counts in the microspore nuclei formed as a result of meiosis. In 130 figures counted, RANDOLPH (1941) found 116 with one B-type and 14 with none. Since male gametes with two or more B-types are obtained from a 2B plant (as shown by the results of the oB×2B cross), these must have been produced from 1B microspores. The proportion of oB progeny from the oB×2B cross is also greater than would be expected from the microspore counts. This suggests that oB gametes are also derived from 1B microspores.

The indication that both oB and 2B gametes are obtained from 1B microspores suggests the possibility that the B chromosome is undergoing "mitotic nondisjunction" in one, perhaps rarely in both, of the divisions which produce

[1] This investigation was begun and largely completed at the UNIVERSITY OF MISSOURI, Columbia, Missouri.

the gametes. By this mechanism, a B chromosome would be distributed in such a way that one of the daughter nuclei would receive two B-types and the other would receive none. The result of the oB×1B cross lends support to this view. Although a sizeable number of plants produced by this cross received two or more B chromosomes, the average number of B-types per plant was approximately 0.5. This may be interpreted to mean that the production of 2B gametes from 1B microspores is balanced by the production of a numerically

TABLE 1

Distribution of B chromosomes among progeny of crosses between oB and 1B or 2B parents.

NUMBER OF B-TYPES IN PARENTS	B-TYPE NUMBER IN F_1					NUMBER OF F_1 PLANTS	AVERAGE NO. B-TYPES PER F_1 PLANT	INVESTIGATOR
	0	1	2	3	4			
0×1	76	22	15			113		Longley 1927
	32	13	5	2		52		Randolph 1941
	108	35	20	2		165	.49	
0×2	22		37		2	61	1.34	Randolph 1941
1×0	109	58	3			170		Longley 1927
	46	19				65		Randolph 1941
	155	77	3			235	.35	
2×0	8	41	3			52	.90	Randolph 1941

equivalent class of oB gametes, as would be expected from the hypothesis of mitotic nondisjunction.

A direct cytological determination of what is happening during the microspore divisions is not feasible for technical reasons. A determination by genetic methods is also precluded since the B chromosome cannot be identified by a phenotypic effect. However, through interchanges with members of the basic set (these will be referred to as A-B interchanges), it should be possible to attach A-type segments carrying known genes to B-type segments and thus to trace the behavior of the latter. This method rests on the assumption that the anomalous behavior of the B chromosome does not depend on its remaining intact. The validity of this assumption is indicated by observations of B-type fragments (RANDOLPH 1941). It should also be possible, by a comparison of the various B-segments produced by breakage at different points in the chromosome, to locate the element within it that is responsible for its aberrant performance.

MATERIALS AND METHODS

Mature pollen from plants with from four to ten B chromosomes was treated with X-rays and applied to silks of plants devoid of B-types. The

radiation was produced by a Coolidge tube operated at 138 K.V.P. and was unfiltered except for the anther wall and the damp paper towelling in which the anthers were wrapped. The doses applied ranged from 600 to 1,600 r-units.

Only a small fraction of the progeny was expected to carry the A-B interchanges. The plants most likely to carry them were screened from the F_1

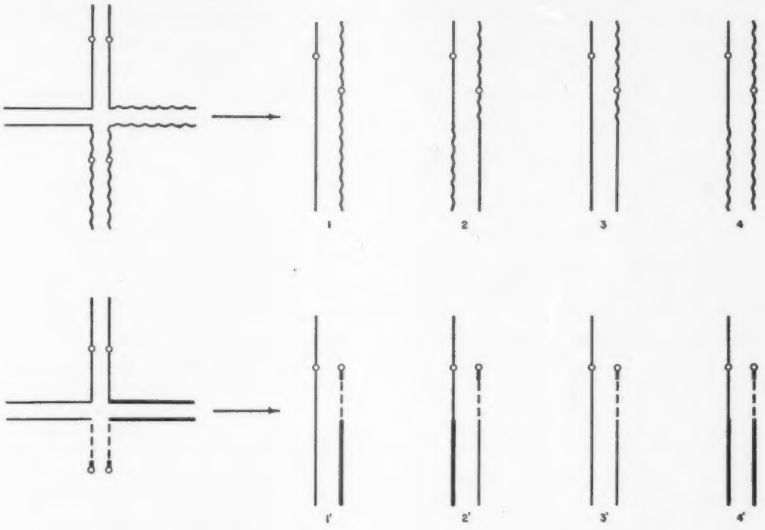

FIG. 1.—Main spore types expected from a plant heterozygous for an A-A interchange (above) compared with those expected from a plant heterozygous for an A-B interchange (below). (Only the chromosomes involved in the interchange are shown.) Note that types 3 and 4 are both deficient for A-chromatin whereas only type 4′ is thus deficient. The respective pachytene configurations are given at the left. The B-type is represented as having a terminal centromere; see p. 405 for a fuller discussion of this.

population by an examination of the mature pollen of each plant. Normal maize plants in healthy condition produce pollen with nearly all grains uniform in size and well-filled with starch. There are usually a few defective grains present, seldom as high as five percent. Plants heterozygous for a deleterious deficiency produce normal and defective grains in approximately equal numbers. Plants heterozygous for an interchange between A-type chromosomes (A-A interchanges) usually yield the same pollen picture since two of the four main types of spores formed are deficient for A-chromatin (fig. 1). On the same basis, plants heterozygous for an A-B interchange would be expected to produce 25 percent of defective pollen since one of the two deficient spore types is deficient only for B-chromatin and this does not alter pollen appearance. This spore type also carries a duplication for A-chromatin but duplications ordinarily have no visible effect on pollen development.

The A-B interchanges could therefore be distinguished by pollen examination from deficiencies and most A-A interchanges. There are, however, some A-A interchanges in which one of the deficient spore types produced by the heterozygous plant develops into a visibly normal pollen grain with the result that only 25 percent of the grains are defective in appearance. Examples of this type, involving the satellite of chromosome 6, have been reported by BURNHAM (1932) and CLARKE and ANDERSON (1935). Plants heterozygous for an inversion also produce defective pollen, in proportions depending on the amount of crossing over within the inverted region. Microsporocyte samples were taken from the F_1 plants and were stored until after pollen examination. The identification of those plants heterozygous for an A-B interchange, among the 25 percent group, was accomplished by cytological examination.

The presence of an A-B interchange could be detected either in pachytene or in diakinesis. In pachytene, the interchange, in heterozygous condition, assumes the familiar cross-configuration if an intact B-type is present. If an intact B-type is not present, a T-configuration is frequently formed, as a result of the homologous pairing of the A-segments and the nonhomologous pairing of the B-segments. In either case, the interchange complex may be detected readily owing to the singular pachytene appearance of the B-type (MCCLINTOCK 1933). Plants heterozygous for an A-B interchange show, in diakinesis, nine A-bivalents and the interchange complex, in contrast with plants heterozygous for an A-A interchange, in which eight bivalents are formed. The A-B interchange complex is in a chain configuration if an intact B-type is not present. If it is present, it usually associates with the complex to form a ring configuration. This offers especially convincing evidence of an A-B interchange since B chromosomes ordinarily do not pair with members of the basic set. When two or more extra B-types are present, some or all of them often associate with the complex in multivalent pairing; otherwise, they form separate univalents, bivalents, or multivalent groups.

EXPERIMENTAL RESULTS

Eight A-B interchanges were obtained from approximately 500 plants screened by the method just described. Chromosomes 1 and 7 each were involved in two interchanges and chromosomes 4, 6, and 9 each were involved once. In one of the interchanges, the A chromosome has not yet been identified.

This report deals only with the interchange involving chromosome 4, designated as TB-4a. The investigation of the other interchanges is still in a preliminary stage. It should be noted, however, that in five of the latter, the investigation has proceeded far enough to indicate that the behavior described for TB-4a applies to these as well.

In the case of TB-4a, the point of interchange in chromosome 4 is in the short arm, approximately one-eighth of the distance from the centromere to the end of the arm. In the B-type, the point of breakage is at or very near the junction of the euchromatic and the main heterochromatic segments (fig. 2). Approximately 25 percent of the pollen grains produced by a plant heterozy-

gous for TB-4a are devoid of starch. The pollen grains of the homozygote are normal in appearance.

Evidence of mitotic nondisjunction

The hypothesis of mitotic nondisjunction, set forth to account for the anomalous transmission of the B-types, has different consequences depending on whether nondisjunction occurs in the first or the second division of the microspore. The formation of the male gametes in maize proceeds as follows. The microspore nucleus derived from the meiotic divisions divides to form a generative and a vegetative, or tube, nucleus. The generative nucleus divides again to form the two gametic nuclei. Thus the mature pollen grain is trinucleate; it carries two crescent-shaped sperm and the tube cell (WEATHERWAX 1917). The latter presumably controls the growth of the pollen tube down the

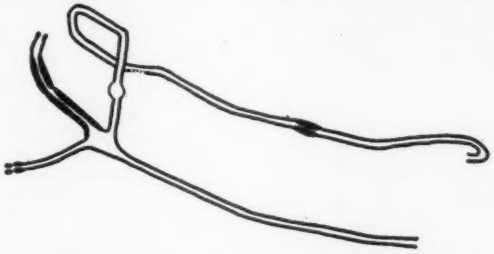

FIG. 2.—Camera lucida drawing of TB-4a in heterozygous condition in the pachytene stage of the microsporocyte.

silk and is not directly involved in the fertilization process. One of the gametic nuclei fuses with the polar nuclei in the embryo sac to form the primordial endosperm nucleus. The other gamete fertilizes the egg.

If, starting with a 1B nucleus, nondisjunction occurs only in the first division of the microspore, either the generative nucleus or the tube nucleus would receive two B-types and the other would receive none. If the generative nucleus receives the two B-types, the gametic nuclei derived from it would each have two B-types. If the generative nucleus does not receive the B-types, the gametes would also lack them. In either case, the two gametes within a single pollen grain would be identical with respect to B chromosome number. If, on the other hand, mitotic nondisjunction occurs only in the second division of the microspore, both the generative and the tube nuclei would have one B-type. The gametic nuclei of a pollen grain in this case would not be alike in B chromosome number; one would receive two B-types and the other would receive none.

In appropriate crosses, fertilization involving male gametes that are alike in B chromosome number would produce a seed with an endosperm and embryo also alike in this respect. If the gametes of a pollen grain are not alike, the endosperm and embryo would also differ. From a $0B \times 2B$ cross, for example, we would expect from nondisiunction in the first division of the micro-

spore that the embryo and endosperm would both contain either two B-types or none. If nondisjunction occurs in the second division, we would expect some of the seeds to have a 2B endosperm and a oB embryo and others to have a oB endosperm and a 2B embryo, depending on the manner in which the oB and 2B gametes of the pollen grain are engaged in fertilization. The time of nondisjunction could therefore be fixed by an examination of these tissues; unfortunately, the endosperm does not lend itself readily to cytological study.

Crosses involving TB-4a were made to test the hypothesis of mitotic non-

TABLE 2

Results obtained when Su Su plants homozygous for TB-4a were crossed as seed parents with normal su su plants.

CROSS	TOTAL SEEDS	SUGARY SEEDS
34.3—2×6.1—1	228	0
34.4—4×6.1—1	165	0
35.1—1×3.4—4	84	0
35.1—2×6.2—6	285	0
35.2—7×6.1—1	244	0
35.2—8×6.1—1	151	0
Totals	1,157	0

disjunction and to decide between the foregoing alternatives. Plants homozygous for the interchange were intercrossed with plants that were normal and lacked B chromosomes. (These crosses are analogous to the oB×2B and 2B×oB crosses insofar as B-chromatin is concerned except that the B-types involved in the interchange are not intact.) The normal parent was homozygous for *su* (sugary endosperm), a recessive gene located in the short arm of chromosome 4 (ANDERSON and RANDOLPH 1945). The interchange parent carried the dominant allele, *Su*, in homozygous condition.

The results of these crosses were widely different depending on whether the interchange parent served as the male or the female. When TB-4a was transmitted by the seed parent, all of the F_1 seeds were nonsugary (table 2), as would be expected from normal behavior. When the interchange parent served as the pollen source, approximately half of the F_1 seeds were sugary (table 3).

The sugary seeds obtained from the latter cross were interpreted as indicating a deficiency in the endosperm of the interchange chromosome bearing the *Su* gene. These seeds were grown for a cytological examination of the microsporocytes to determine the chromosomal content of the embryo. Twenty-four plants were examined and each was found to have an extra B^4 chromosome, the interchange chromosome with a B-type centromere. They contained two B^4 chromosomes in addition to a normal chromosome 4 and a 4^B chromosome (the interchange chromosome with the chromosome 4 centromere).

It remained to be determined whether the chromosome that is absent in the endosperm of the sugary seeds is the same one that is present as a duplica-

tion in the hyperploid embryo. This was accomplished by using the hyperploid plants as seed parents in crosses with normal plants that were homozygous for *su*. It was observed in diakinesis of the hyperploids that in a majority of the cells, the normal chromosome 4 paired with the 4^B chromosome and the two

TABLE 3

Results obtained when Su Su plants homozygous for TB-4a were crossed as pollen parents with normal su su plants. (34.3–7 had no intact B-types; 34.4–7 had two intact B-types.)

CROSS	TOTAL SEEDS	SUGARY SEEDS	
		%	χ^{2*}
3.3–1×34.3–7	222	48.2	.16
6.2–3×34.3–7	166	44.0	2.4
6.2–6×34.3–7	270	55.2	2.9
78.1A–2×34.3–7	278	48.6	.23
78.1–2×34.4–7	201	53.2	.84
7.2–2×34.4–7	209	57.9	5.2
7.3–3×34.4–7	200	48.0	.32
8.4–3×34.4–7	245	57.6	5.6
Totals	1791	51.9	2.7

* Calculated for deviation from 50%.

TABLE 4

Results of crosses involving hyperploid plants for location of Su with respect to breakage point in chromosome 4. See text for description of cross.

TOTAL SEEDS	SUGARY SEEDS %
150	5.3
234	5.1
287	5.9
220	11.4
300	5.3
266	5.6
305	3.3
158	4.4
261	11.1
Totals 2,181	6.4

B^4 chromosomes formed a separate bivalent. In some cells, all four chromosomes were associated in a single complex. It would be expected from these observations that two types of spores would predominate, one containing 4^B and B^4 and the other containing chromosome 4 and B^4 (fig. 3). If *Su* is on 4^B, we would expect, since the normal chromosome 4 carries *su*, that half of the seeds obtained from the cross would be sugary. If *Su* is located on B^4, each of the two main types of spores would have this allele and only a small percentage of sugary seeds would be expected, these resulting from meiotic disjunction

of the relatively rare complex association. The results of the cross (table 4) show that *Su* is on the B^4 chromosome.

As already indicated, the noncorrespondence in the chromosomal composition of the endosperm and embryo in the sugary seeds is evidence of a parallel noncorrespondence in the composition of the gametes contained in the pollen grains furnished by the parent homozygous for TB-4a. Thus the gametic nucleus that fused with the polar nuclei to form the primary endosperm nucleus was deficient for the B^4 chromosome and that which fertilized the egg carried this chromosome in duplicate. This means that nondisjunction of the B^4 chromosome occurred in the division of the generative nucleus. Figure 4

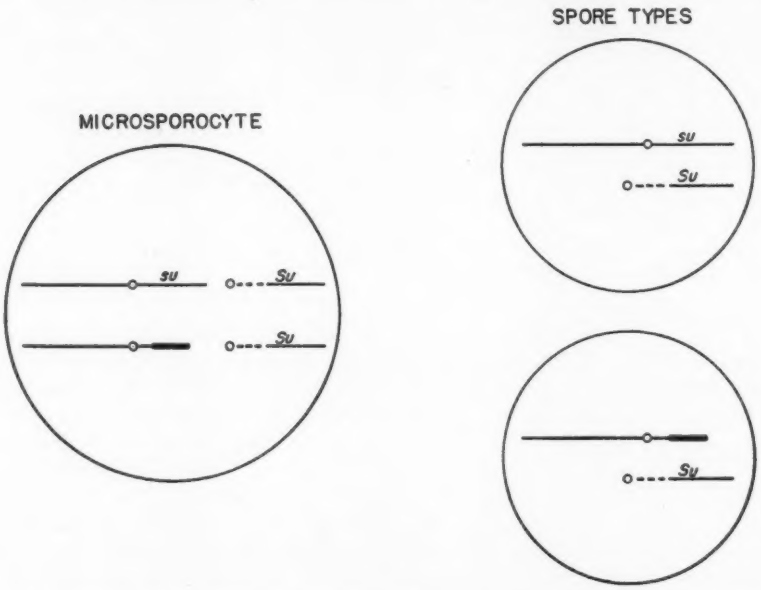

FIG. 3.—Association of chromosomes in diakinesis of microsporocyte and expected spore types.

illustrates the probable course of events in the formation of a pollen grain on the basis of nondisjunction in this division.

Another type of seed is also expected as a result of mitotic nondisjunction. If the gamete deficient for the B^4 chromosome fertilizes the egg and the gamete carrying two doses of this chromosome fuses with the polar nuclei, we should obtain seeds that are nonsugary and have a deficient embryo. Twenty-seven plants grown from nonsugary seeds included twenty-four that were deficient for the B^4 chromosome and otherwise carried a normal chromosome 4 and 4^B. The remaining three were simply heterozygous for the interchange, as would be expected from normal disjunction in the division of the generative nucleus.

In summary, when a plant homozygous for TB-4a and used as the pollen

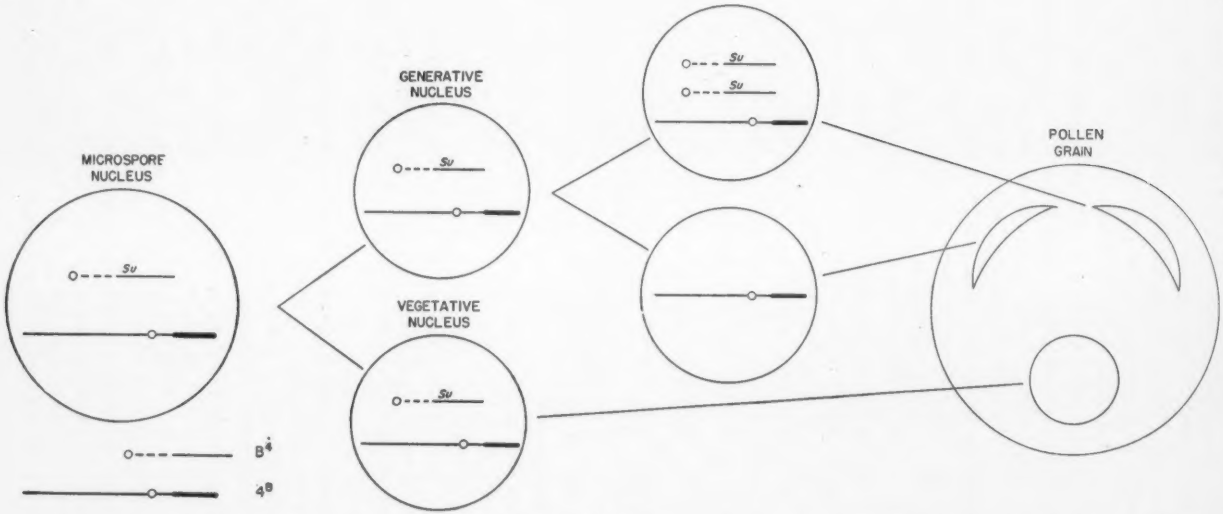

FIG. 4.—Probable course of events in the development of the pollen grain in which nondisjunction has occurred in the division of the generative nucleus.

parent is crossed with a normal plant, three types of seeds are produced. One of these has a hyperploid embryo and a deficient endosperm. Another has a deficient embryo and a hyperploid endosperm. The third type, which does not occur as frequently as the other two, has a euploid embryo, heterozygous for the interchange, and presumably also a euploid endosperm. The first two types are the result of mitotic nondisjunction of the B^4 chromosome in the second microspore division; the last, of normal disjunction.

If mitotic nondisjunction occurs in the development of the female gametophyte, its products are not transmitted to the viable eggs. When TB-4a was transmitted by the eggs in the cross of TB-4a (*Su Su*) × Normal (*su su*), none of the F_1 seeds were sugary (table 2). Twenty-one of the seeds were grown and all were found to be simply heterozygous for the interchange. It may be that the occurrence of nondisjunction, in the development of the female gametophyte, produces an inviable gamete which prevents seed formation or results in a seed lacking an embryo. However, there was no marked evidence of the elimination of inviable gametes on the ears obtained from this cross and all of the seeds had normal-appearing embryos. If mitotic nondisjunction occurs at all in the formation of the egg, it is infrequent compared with the rate with which it occurs in the second division of the microspore.

Rate of mitotic nondisjunction

The three types of seed obtained from the cross described in the preceding section are referable to three types of fertilization (fig. 5). Types I and II are reciprocals and involve male gametes that are aneuploid as a consequence of mitotic nondisjunction. In type III, the male gametes are euploid and are the product of normal disjunction.

The rate of nondisjunction in the division of the generative nucleus may be estimated from the data in table 3 if two assumptions are made. One is that the fertilization types I and II occur in equal numbers. The second assumption is that the three types of seed are equally viable. The ears obtained from the cross carried a full set of seed and the sugary and nonsugary seeds were normal in appearance. There was no gradation in seed size to suggest a possible elimination of either kind.

The consequence of each occurrence of mitotic nondisjunction would be either a sugary or a nonsugary seed and, on the first assumption, either type would be equally probable. The frequency of sugary seeds would thus be half of the rate of occurrence of mitotic nondisjunction. If the latter were 100 percent, that is, if it occurred in all of the second microspore mitoses, 50 percent of the seeds obtained from the cross should be sugary. The sugary seeds comprised 51.4 percent of the total population obtained from eight crosses involving two different interchange parents (table 3). By the chi-square test, this value does not deviate significantly from 50 percent but the deviation from 45 percent appears to be significant. It would seem, therefore, that the rate of mitotic nondisjunction is very high, between 90 and 100 percent. The evidence that it is not 100 percent has already been presented; three of the

twenty-four plants grown from nonsugary seeds were euploid and heterozygous for the interchange and were therefore the products of normal disjunction.

The calculation of the rate of nondisjunction must be made with some reservation. In two of the crosses listed in table 3, the chi-square values, 5.2 and 5.6, suggest that the deviations from 50 percent may not be due to sampling error alone. The same pollen parent was used in both crosses but it was also used in two other crosses, made under comparable conditions, which did

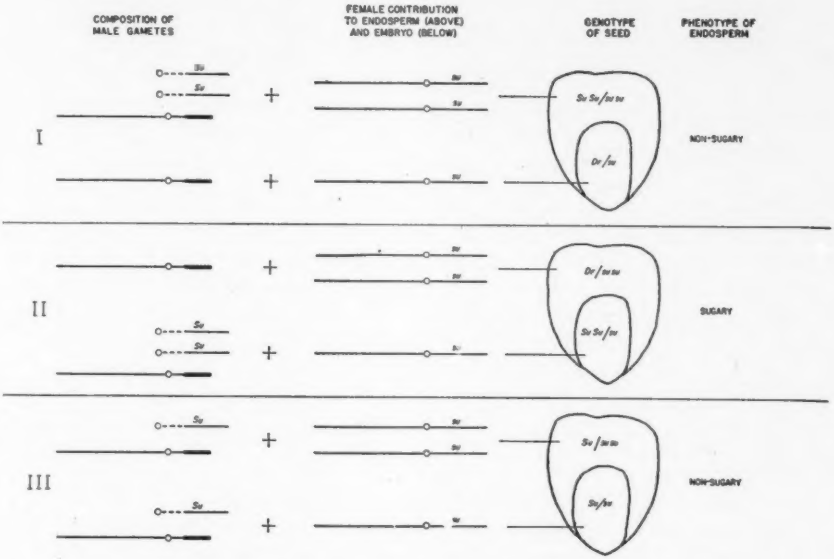

FIG. 5.—Types of double-fertilization as a result of mitotic nondisjunction (I and II) and normal disjunction (III). Types I and II are reciprocals.

not give a marked deviation. This suggests an influence of the female parent on the sugary seed frequency.

If a significant deviation from a 50 percent segregation of sugary seeds persists in additional crosses, the assumption that fertilization types I and II occur with equal frequency will require revision. Otherwise, another source of sugary seeds, not indicated by the results so far obtained, will be required. The excess of sugary seeds in some crosses suggests that fertilization type II occurs more frequently in these crosses than type I. The ratio of these two types cannot be determined directly from seed counts, since nonsugary seeds are derived from both types I and III. These could be separated if the seeds were grown and the resulting plants examined for the presence or absence of chromosome B^4. This experiment is in progress.

Stability of the B^4 chromosome during endosperm development

In view of the high rate of mitotic nondisjunction of the B^4 chromosome in the division of the generative nucleus, it would be of interest to test for its occurrence in other divisions. Evidence has already been presented that mitotic nondisjunction is rare or absent in the divisions of the female gametophyte that lead to the formation of the egg. The study can be extended to the endosperm through the use of the sugary character.

The endosperms of the nonsugary seeds produced by the cross of Normal (*su su*) × TB-4a (*Su Su*) and its reciprocal are expected to be of three types, with respect to the sugary gene. When the interchange is transmitted by the seed parent, the expected composition of the endosperm is *Su Su su*. When it is transmitted by the pollen parent, two endosperm types, *su su Su Su* and *su su Su*, are obtained (fig. 5). The B^4 chromosome carries *Su* and the normal chromosome 4 carries *su*.

The occurrence of mitotic nondisjunction of the B^4 chromosome in the first division of a primary endosperm nucleus of composition *su su Su* would result in a sugary sector deficient for this chromosome in an otherwise nonsugary seed. Assuming uniform endosperm development, such a sector would comprise half the area of the endosperm. Smaller sectors would be obtained from mitotic nondisjunction in later divisions. In either case, the relationship between the frequency of sectors and the rate of nondisjunction would be a simple one; each occurrence of nondisjunction should yield a sugary sector.

The relationship is much more complicated in the seeds which have two B^4 chromosomes in the endosperm. The "loss" of both of these would be a requirement for the occurrence of a sugary sector. It is conceivable that the loss could occur in either of two ways as a consequence of nondisjunction. First, one of the B^4 chromosomes could undergo nondisjunction and the other disjoin normally to produce daughter cells with three and one B^4 chromosomes, respectively. The recurrence of nondisjunction in a subsequent division of the cell containing one B^4 chromosome would yield a cell line deficient for this chromosome, hence a sugary sector. Or, secondly, the B^4 chromosomes might undergo nondisjunction simultaneously, with either of two results. If the B^4 chromosomes move to one pole, the daughter cells would have four B^4 chromosomes in one and none in the other. If they move in opposite directions, the original condition would be restored in each of the daughter cells. If these alternatives are equally probable, only half of the cases of simultaneous nondisjunction would lead to sugary sectors.

A simple example will illustrate the relationship between the rate of nondisjunction and the frequency of sugary sectors when two B^4 chromosomes are present. A sugary sector comprising half the endosperm area would be expected from half the cases of simultaneous nondisjunction occurring in the division of the primary endosperm nucleus. If x is the rate of nondisjunction for each B^4 chromosome per cell division and if each acts independently, the expected frequency of sectors of this size would be given by $x^2/2$. For a rate of nondisjunction of ten percent (one occurrence per ten divisions), the frequency of

"1/2"-sectors would be 0.5 percent, or five per thousand seeds. Smaller sectors would be expected to occur more frequently owing to a greater opportunity for both simultaneous and recurrent nondisjunction in later divisions. Sectors comprising less than one-sixteenth of the endosperm are detectable (STADLER and SPRAGUE 1936).

The seeds obtained from the crosses between normal (*su su*) plants and plants homozygous for TB-4a (*Su Su*) were examined carefully for sugary sectors. None were found among 1157 seeds produced when the interchange was transmitted by the seed parent. The reciprocal cross yielded seven sectors among 1678 seeds examined. The production of endosperms containing a single B^4 by the latter cross (type III, fig. 5) probably accounts for the difference in the frequency of sugary sectors in the two crosses.

It should be made clear at this point that the occurrence of a sugary sector does not necessarily mean that mitotic nondisjunction of the B^4 chromosome is involved. These sectors occur spontaneously in appropriate crosses involving normal parents, with a frequency of approximately one-tenth of one percent (STADLER and SPRAGUE 1936). They could arise as a result of mutation or the production of deficiencies including the *Su* locus due to fragmentation of the B^4 chromosome. The B-type chromosome is subject to fragmentation (RANDOLPH 1941; DARLINGTON and UPCOTT 1941) and since the B^4 chromosome contains the proximal third of the B-type chromosome, it is not unlikely that this mechanism furnishes at least a fraction of the sugary sectors found. With regard to nondisjunction, the results indicate that the B^4 chromosome is remarkably regular in its behavior in the endosperm mitoses, in contrast with its aberrant behavior in the division of the generative nucleus of the microspore.

DISCUSSION

Mitotic nondisjunction, as demonstrated for the B^4 chromosome, explains the transmission of the intact B-chromosome in most particulars. The exceptional 0B and 2B plants obtained from the 0B×1B and 0B×2B crosses (table 1) have their counterparts in the deficient and hyperploid plants produced by the crosses involving TB-4a as the male parent. The relatively rare 3B and 4B progeny from these crosses, however, cannot be accounted for on the basis of a single occurrence of nondisjunction. They suggest that this process may also occur occasionally during some division other than the second microspore mitosis, possibly in meiosis.

The uniformly high rate of nondisjunction, approximating 100 percent, estimated for the B^4 chromosome does not apply in all cases to the intact B-type. A high rate is evident in the results of the 0B×2B cross, in which no 1B progeny, indicative of normal disjunction, were obtained. In the 0B×1B cross, however, a considerable number of 1B plants were produced and a good match with the observed data can be obtained if a rate of nondisjunction of only 50 percent is assumed (table 5). The calculations in this table are made on the basis that 0B and 1B microspores are produced in equal numbers by the 1B parent. This assumption affects the proportion of the 0B progeny in the F_1

population. It does not affect, however, the ratio of the 1B and 2B progeny to each other, and this ratio is in itself a sufficient indicator of the rate of nondisjunction.

It is unlikely that the rate of nondisjunction is influenced by whether the parent has one B-type or two, since in either case the microspore in which nondisjunction occurs has one B-type. Different rates are found for different plants of the same B chromosome number, as is shown by the results of five 0B×1B crosses, each involving a different 1B parent (LONGLEY 1927). One of these crosses yielded eight plants with two B-types and none with one (100 percent nondisjunction) and another gave none with two B-types and nine with one (no nondisjunction). The effect of environmental variation on the rate of nondisjunction is not known. There is a possibility that we are dealing

TABLE 5

Comparison of data from 0B×1B crosses with distribution expected from various rates of mitotic nondisjunction.

	B CHROMOSOME NUMBER IN F_1			
	0	1	2	3
Observed*	108	35	20	2
Expected from:				
50% nondisjunction	103	41	21	
75%	114	21	31	
100%	124	0	41	

* Combined data of LONGLEY and RANDOLPH.

with two or more types of B chromosomes (a view held for different reasons by DARLINGTON and UPCOTT 1941) which differ in their capacity for normal disjunction. It is also possible that variations in the basic genotype are responsible for these differences in the rate of nondisjunction. The work with TB-4a (and several other A-B interchanges) has not shown these differences to a marked extent but the results are not extensive enough to warrant the conclusion that they do not occur.

Localization of the chromosomal element responsible for mitotic nondisjunction

Fragment chromosomes derived from the B-type chromosome by terminal deletion give transmission results that are similar to those obtained for the intact B-type itself (RANDOLPH 1941). The smallest of these is the "F" chromosome which consists "merely of a centromere with an almost insignificant amount of attached chromatin." The evidence that this diminutive chromosome undergoes nondisjunction suggests that the B-type centromere is responsible for the aberrant process.

Similar evidence has been obtained from the investigation of the A-B interchanges. In the case of TB-4a, the interchange chromosome which undergoes mitotic nondisjunction carries the centromere and the proximal third or so of the B-type chromosome. The other interchange chromosome, carrying the

distal two-thirds of the B-type, disjoins normally. The study of five other A-B interchanges has proceeded far enough to show that the same relationship holds in these as well; the aberrant chromosome in each case is that one which possesses the B centromere.

In these interchanges, the points of breakage in the B-type chromosome range from one which occurred near the end of the chromosome to another well within the proximal euchromatic segment. The size of the A-segment in the B^A chromosome also varies widely. In one interchange, the A-segment consists of only the tip of the long arm of chromosome 7. In another, almost all of the short arm of chromosome 1 is involved, to form an interchange chromosome which is longer than the shorter members of the basic set. The length of the chromosome undergoing mitotic nondisjunction evidently has little or nothing to do with determining the occurrence of this process.

Other examples of mitotic nondisjunction are found in rye (HASEGAWA 1934; MÜNTZING 1946), in sorghum (DARLINGTON and THOMAS 1941), and in the fungus gnat *Sciara* (METZ 1938). In these, as in maize, nondisjunction occurs for the most part only in certain mitoses. The supernumerary in rye undergoes nondisjunction in the first microspore mitosis and also at some point in the development of the female gametophyte. In sorghum, nondisjunction occurs in a supernumerary division of the vegetative nucleus of the pollen grain. In *Sciara*, it occurs in the division of the secondary spermatocyte. In the four cases, a specific chromosome (or a derivative of this chromosome) undergoes mitotic nondisjunction; the other chromosomes of the set disjoin normally. Thus there are two conditions which are essential to the occurrence of mitotic nondisjunction: (1) a chromosome of a particular kind, and (2) a mitosis that imposes a restriction to normal disjunction that is specific for this chromosome.

A comparison of the aberrant chromosomes in the examples cited above reveals a striking relation with respect to the position of the centromere. The centromere in rye is subterminal and in maize it is terminal or nearly so. In *Sciara*, the position is different in different species. It is at or near the end of the chromosome in *S. coprophila* whereas it is median in *S. pauciseta* (SCHMUCK 1934). The aberrant chromosomes in sorghum, as reported in two plants, also have median centromeres and are of two distinct sizes (DARLINGTON and THOMAS 1941). The larger of these has been identified as an iso-chromosome. It has been shown in rye that large and small isochromosomes are derived from the standard supernumerary which has a subterminal centromere (MÜNTZING 1944). The aberrant chromosomes in sorghum (and perhaps also those with median centromeres in some species of *Sciara*) may have been similarly derived. Thus, in each case, the chromosome that is undergoing mitotic nondisjunction either is or may have originated from a chromosome with a subterminal or terminal centromere, in contrast with the normal chromosomes of the respective sets (again expecting *Sciara*) which have distinctly internal, usually median or submedian, centromeres.

There is some argument concerning the position of the centromere of the B-type in maize. MCCLINTOCK (1933) described it as terminal on the basis of examination in the pachytene stage of the microsporocyte. RANDOLPH (1941)

reported a diminutive short arm, seen in pachytene, which was not evident in all figures. He believes that the centromere assumes a spurious terminal appearance in some figures because the short arm folds back against the pycnotic knob adjacent to the centromere in the long arm. DARLINGTON and UPCOTT (1941) reported a sizeable short arm, long enough to permit the formation of observable chiasmata. They suggest that the B-type may vary structurally to give various positions for the centromere. The B-types involved in the A-B interchanges all appear to have terminal centromeres when examined in pachytene and no marked structural differences were found. It is however possible that a very small arm, such as RANDOLPH observed, could have been missed in the examinations.

The similarity between the aberrant chromosomes of the different organisms suggests a relationship between mitotic nondisjunction and the position of the centromere. A chromosome with a subterminal or terminal centromere could be derived from a chromosome with a more centrally located centromere in one of two ways, either by rearrangement of the chromosome or by loss of a segment in one arm. Either case would require a break at or near the centromere. An aberrant chromosome might be produced if, in the process of rearrangement or loss, there is an impairment of the effectiveness of the centromere or adjacent chromatin vital to normal disjunction. If this were the case, it should be possible to produce a chromosome showing mitotic nondisjunction as a result of such structural changes in a normal chromosome.

The telocentric chromosome studied by RHOADES (1940) in maize may represent a case of this kind. This chromosome was found in a plant obtained from a cross involving a parent trisomic for chromosome 5. It consists of the entire short arm of chromosome 5 and has a centromere that is unquestionably terminal. When plants hyperploid for the telocentric chromosome are crossed with normal diploids, the progeny include hyperploid plants of two types. One of these contains a single telocentric chromosome in addition to the diploid complement; the other has two doses of the telocentric chromosome in the form of an isochromosome. These two types are produced in about equal numbers when the male parent transmits the telocentric chromosome. When it is transmitted by the female parent, the progeny containing an isochromosome comprise only 0.57 percent of the hyperploid types. The formation of isochromosomes from telocentric chromosomes is thus much more frequent in the male germ line than in the female germ line.

This dependence on sex is remarkably similar to that found for the occurrence of mitotic nondisjunction of the B-type and the B^4 chromosomes. The tests made by RHOADES show that the formation of the isochromosome is a postmeiotic process. In the light of the behavior of the B^4 chromosome, the results obtained for the telocentric chromosome may be interpreted as another case of nondisjunction in the division of the generative nucleus. The results differ in one respect from those obtained for the B^4 chromosome. In the case of the latter, two chromosomes are distributed as individuals to one of the gametes. The telocentric chromosome, however, forms an isochromosome in the process. It is doubtful that nondisjunction occurs first and is followed by

a fusion of the terminal centromeres since RHOADES found that two telocentric chromosomes could exist in one nucleus without forming an isochromosome. It would seem, rather, that the centromere of the telocentric chromosome either fails to divide or divides tranversely (DARLINGTON 1940) to produce the isochromosome. In this respect, the telocentric differs from both the B-type and the B^4 chromosomes. In all three cases, however, it is probable that the centromere is responsible for the aberrant behavior.

Cytological observations by MÜNTZING (1946) in rye suggest that the centromere may not be the only chromosomal element responsible for mitotic nondisjunction. The aberrant chromosome in rye lags in the first microspore mitosis and is usually included in the generative nucleus. The lagging is due to the failure of a region near the centromere to divide in concert with the rest of the chromosome. The activity of the centromere appears to be normal

Some other uses of A-B interchanges

The A-B interchanges provide a means of obtaining aneuploid plant types that are difficult to produce by other methods. The duplications and deficiencies that are obtained in this way are useful in the study of various cytogenetic problems. The examples that follow show some uses of the aneuploid types in problems under investigation.

It has been shown in the case of TB-4a that functional deficient and hyperploid male gametes are produced by mitotic nondisjunction in the division of the generative nucleus. As a result, progeny are obtained which carry a specific segment of chromosome 4 in one, two, and three doses. It should be possible, by appropriate crosses, to obtain the segment in even higher numbers. For example, a plant hyperploid for the B^4 chromosome forms hyperploid eggs. If these are fertilized by sperm that are also hyperploid for the B^4 chromosome, plants should be obtained that carry four doses of the segment of chromosome 4. The limit to continued accumulation would depend only on the degree of unbalance caused by the segment. Preliminary results have shown that a dosage series may also be established in the case of other A-B interchanges as well. The mechanism of mitotic nondisjunction thus provides a method for the study of the effect of specific chromosomal segments in various numbers. In the same way, it makes possible a determination of the effect of known recessive or dominant genes located in these segments.

The production of functional deficient gametes may also be utilized to locate recessive genes within the chromosome. A plant which contains the recessive gene is crossed as the seed parent with a plant carrying an A-B interchange and homozygous for the dominant allele. The deficient progeny will show the recessive character if the locus of the gene is distal to the point of breakage in the A chromosome. This method is illustrated by the use of TB-4a in locating *su*. The TB-4a parent was homozygous for *Su*. Crosses with *su su* plants gave kernels that were of the sugary phenotype. These kernels were deficient for the B^4 chromosome thus indicating that *Su* is carried on this chromosome.

Intercrosses involving different A-B interchanges may provide information

as to whether or not maize is of polyploid origin. In appropriate crosses, plants can be produced that have two different A-segments in duplication. For example, a cross between a plant that has TB-4a with one that has TB-7a will yield plants in which the B^4 and the B^7 chromosomes are present as duplications. If the segment of chromosome 4 has a region within it that is a duplication of a region in the chromosome 7 segment, the homology might be revealed by the regular synapsis of these regions.

Another interesting aspect of these interchanges is the possibility of an interaction between the heterochromatin of the B-type and the adjacent A-chromatin in the interchange chromosomes. Drosophila investigations have shown that the action of a gene may be altered when, following translocation, it is brought adjacent to or relatively near a heterochromatic region. Such an effect has not been observed in maize although many interchanges between A chromosomes have been studied. No marked effect has been apparent in the case of TB-4a, but the break in the B-type in this interchange is probably not within the heterochromatic segment. In other A-B interchanges, the B-type was broken well within this segment with the result that B heterochromatin is in direct contact with A euchromatin. Interchanges of this kind provide material for a study of the heterochromatin-euchromatin relationship in maize.

SUMMARY

Eight interchanges between A-type and B-type chromosomes were obtained from B-bearing pollen treated with X-rays. The behavior of one of these, involving chromosome 4 of the basic set, is reported in this paper.

The results of crosses involving plants homozygous for this interchange (designated TB-4a) show that one of the interchange chromosomes (B^4) undergoes nondisjunction in the second division of the microspore. As a consequence, the two gametes of a pollen grain carry different chromosomal complements. One has the aberrant chromosome in duplicate; the other is deficient for this chromosome. Both gametes are functional.

Nondisjunction of this chromosome occurs in most but not all of the second microspore mitoses. The seeds obtained from a cross between a pollen parent homozygous for TB-4a and a normal seed parent are therefore of three kinds. One has an endosperm that is deficient for the B^4 chromosome and an embryo that carries the chromosome in duplicate. Another has a hyperploid endosperm and a deficient embryo. The third has a euploid endosperm and is simply heterozygous for TB-4a in the embryo.

The B^4 chromosome possesses the centromere and proximal third of the B-type chromosome. The interchange chromosome bearing the centromere of chromosome 4 disjoins normally. Preliminary studies in five other A-B interchanges have shown that it is the interchange chromosome which bears the B-centromere that is aberrant in each case.

The B^4 chromosome is relatively stable in endosperm development and undergoes nondisjunction rarely, if ever, in the development of the egg.

The anomalous male transmission of the intact B-type chromosome may

also be accounted for on the basis of nondisjunction in the second microspore division.

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INHERITANCE IN THE CARNATION, DIANTHUS CARYOPHYLLUS. IV. THE CHEMISTRY OF FLOWER COLOR VARIATION, I[1]

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STUDIES on the relationship between the genetical factors for flower color and the structural variations in the pigments responsible for the colors have given promise of being one of the most useful experimental methods for the investigation of many important questions concerning certain aspects of the action and interaction of genes. Since WHELDALE and BASSET (1913, 1914) first called attention to the possibilities of the method and performed some of the first experiments, advances have been rapid, and great impetus was given to such studies by the final elucidation of the structures of most of the common anthrocyanins and the development of rapid and accurate methods for their recognition by ROBINSON and ROBINSON (1931, 1932, 1933, 1934). Excellent summaries of the results obtained up to about 1940 have recently appeared (SCOTT-MONCRIEFF, 1938; LAWRENCE and PRICE, 1940) and no more than a brief review of certain of the findings will be included in the present discussion.

By far the greatest attention has so far been paid to the genetics of structural variations in the anthocyanin[2] pigments and relatively little exact information is available concerning possible similar variations in the anthoxanthins[3] which almost invariably accompany the anthocyanins in the cell sap and which bear a close structural relationship to them. Studies carried out by BEALE, *et al.* (1939) on *Lathyrus odoratus*, HAGIWARA (1931, 1932) on *Pharbitis nil*, LAWRENCE and SCOTT-MONCRIEFF (1935) on *Dahlia variabilis*, SANDO, *et al.* (1935) on *Zea mays*, BEALE, *et al.* (1940) on *Verbena*, WIT (1937) on *Callistephus chinensis*, and SCOTT-MONCRIEFF on *Primula acaulis* (1932) and *Cheiranthus cheiri* and *Tropaeolum majus* (1936), have shown that certain structural variations in the anthocyanin pigments may be regarded as more or less direct expressions of the activity of particular genes. Among these effects are those brought about by genes which control

[1] The present article presents the results of the first part of a study which because of the war had to be suspended. As there may be some time before full scale resumption of the work can be undertaken it has been deemed desirable to publish the results obtained to date.

[2] "Anthocyanin" is the term applied to the naturally-occurring glycosides of the polyhydroxy-2-phenylbenzopyrilium salts which comprise the class of plant pigments responsible for most of the blue to red colors of flowers and fruits.

[3] "Anthoxanthin" is a rather general term applied to sap-pigments other than those of the anthocyanin type. It refers in most cases to flavone derivatives.

(a) the degree of oxidation (hydroxylation) of the 2-phenylbenzopyrilium nucleus,
(b) the methylation of one or more of the hydroxyl groups in the 2-phenyl nucleus,
(c) the number of sugar residues associated with the anthocyanidin nucleus, and their points of attachment,
(d) the concentration of the anthocyanin in the cell-sap,
(e) the pH of the cell-sap,
(f) the presence or absence of co-pigments.

In most of the cases so far examined two or more (complementary) factors have been recognized as necessary for anthocyanin formation.

Few combined genetical and chemical data are available which show a corresponding factorial control of the concentration and structure of the anthoxanthin pigments. Anthoxanthins modify the colors of anthocyanin-pigmented flowers, the nature of the modifying effect being dependent upon the nature of the anthoxanthin and of the anthocyanin and upon their relative concentrations. The bulk of what is known concerning genetical control of anthoxanthin formation is the result of attempts to correlate such "co-pigmentation" effects with factorial differences. Much of this information is unfortunately inexact and some of it has been shown by later work to be erroneous. The chief source of such error or uncertainty appears to be the assumption, which has sometimes been made, that the isolation of a given anthoxanthin (flavone or flavonol) from a certain flower is evidence for the presence of the same anthoxanthin in other flowers, including different color variations, of the same species. There appears to be little justification for such an assumption and no *a priori* reason why the structure of the flavones or flavonols present in a series of color variations of a given species should remain constant from one genotype to another. Indeed, the very nature of the detailed evidence concerning structural variations of the anthocyanins in a series of related genotypes would make it a more reasonable first hypothesis that a constancy of structural type in the anthoxanthins is not to be expected. Whatever the biogenetic relationship between the anthocyanins and flavone derivatives may prove to be, it is reasonable to suppose, in view of their close structural relationship and the consequent probability that their syntheses are closely interrelated, that the same genetic factors which control the amount and structure of the anthocyanins in a series of color variations may have an influence upon the amount and structure of the accompanying anthoxanthins. The evidence so far available has been insufficient to furnish a factual basis for predicting what this influence will prove to be.

Among the questions which arise in this regard are those dealing with the effect of certain genes, which in the cyanic flowers modify the concentration and structure of the anthocyanins, upon the anthoxanthins in flowers lacking the basic complement of genes necessary for the formation of anthocyanins. Let us suppose for example (fig. 1) that a gene *R* is responsible for the formation of a cyanidin (I) derivative while its recessive allele *r* permits the formation of a pelargonidin (II) derivative only. In two white flowers one carrying

R the other carrying *r* will the anthoxanthin (flavonol or flavone) be related to quercetin (III) (or luteolin (IV)) in the one case and to kampferol (V) (or apigenin (VI)) in the other? Will a gene which affects the concentration of anthocyanins in the cyanic flowers exert a corresponding effect upon the concentration of the anthoxanthins in the acyanic flowers?

The answers to questions such as these will undoubtedly furnish a basis upon which to evaluate and to modify existing ideas concerning the synthetic processes by which the flower pigments and structurally related substances

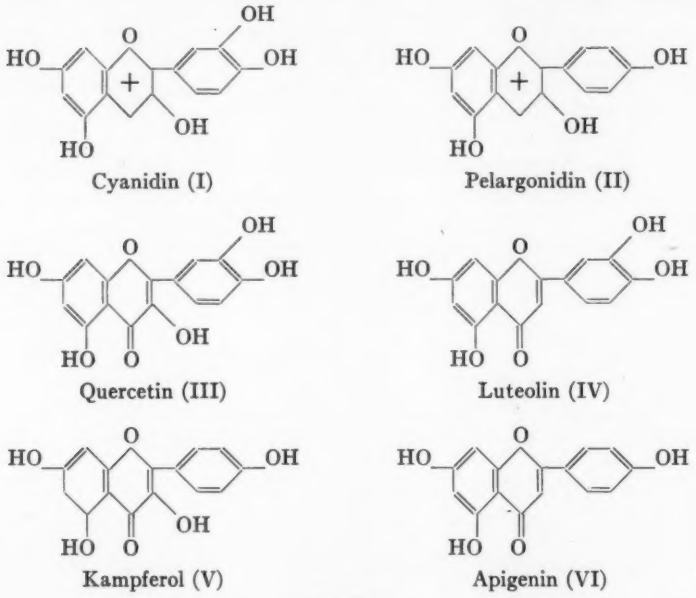

FIGURE 1

are formed. Among the several hypotheses which have been advanced to account for the production of a group of substances so closely related in structure as the anthocyanins and the flavones,[4] two appear to deserve the most serious consideration.

One of these, based largely upon chemical evidence alone (EVEREST, 1918), suggests that the anthocyanins may arise as a result of direct structural modification of a flavone or flavonol by appropriate steps, the last of which is the reduction of a flavonol into the corresponding anthocyanin. The other, resting upon genetical evidence (LAWRENCE and SCOTT-MONCRIEFF, 1935; ROBINSON, 1936), offers the suggestion that the anthocyanins and anthoxanthins arise

[4] The term "flavone" will be used in the generic sense and will frequently refer to flavonols (which are 3-hydroxyflavones) as well.

from a precursor common to both at an early stage but which, in succeeding stages, undergoes modification along two paths, one of which leads to the anthocyanin, the other to the anthoxanthin.

The possibility of direct conversion of flavonol into anthoxyanin is at first sight an attractive one. A comparison of the structures of a representative anthocyanidin (cyanidin (I)) and a typical anthoxanthin (quercetin (III)) shows the simple formal relationship which exists between these classes. The anthocyanidin may be considered as being derived from the flavonol by a reduction of the carbonyl group at C_4 of the latter, a change which gives rise immediately to the anthocyanidin pseudo-base, equivalent, insofar as the state of oxidation is concerned, to the anthocyanidin. It is clear that if any such simple synthetic relationship existed we should expect to find numerous examples of flowers in which the anthocyanin and accompanying anthoxanthin were similarly constituted. Examples of this sort are known but are few in number. SANDO, MILNER and SHERMAN (1935) have found that the purple-husked maize contains a quercetin-3-glucoside and a cyanidin-3-glucoside, and SANDO (1937) has shown that Jonathan apples contain 3-galactosides of both cyanidin and quercetin. EVEREST (1918) has presented inconclusive evidence which indicated that accompanying violanin (a delphinidin derivative) in a so-called black form of the pansy (*Viola tricolor*) is the corresponding flavonol, myricetin. *Rosa gallica*, according to KARRER and SCHWARTZ (1928), contains quercetin-3-rhamnoside and cyanidin-3,5-diglucoside, another example of the correspondence of the aglycones only. In *Antirrhinum majus* both luteolin and apigenin occur in certain color types (WHELDALE and BASSET, 1913, 1914), the related anthocyanidins cyanidin (as a 3-rhamnoglucoside) (SCOTT-MONCRIEFF, 1930) and pelargonidin (as a 2-pentoseglycoside) (SCOTT-MONCRIEFF, 1938) also occurring; but each anthocyanin may be found with either its related or the unrelated flavone. It is to be noted, however, that the identification of the flavones in all of the *Antirrhinum* color-types studied was not carried out. A few other examples showing similar lack of correspondence or partial correspondence make up the bulk of the known cases, and it is important to note, as ROBINSON (1936)has pointed out, that those cases in which complete correspondence of anthocyanin and anthoxanthin have been found have so far been confined to the 3′, 4′-dihydroxy types represented by quercetin and cyanidin, which are by far the commonest structural types of their classes.

The most serious defect in this hypothesis lies in the fact that a lack of correspondence of structural type between the anthocyanin and the flavone derivative in a given flower is the rule, and a more or less complete correspondence, the exception. Numerous specific examples could be cited to show this; a case in point is the observation of BEALE and co-workers (1939) that in *Lathyrus odoratus* the flavonols in highly pigmented types were the same (quercetin and a trace of kampferol) whether the accompanying anthocyanin was derived from pelargonidin, cyanidin or delphinidin (5′-hydroxycyanidin).

Studies on *Dahlia variabilis* by LAWRENCE and SCOTT-MONCRIEFF (1935) have led these authors to suggest that the anthocyanins and anthoxanthins

are derived by parallel rather than by sequential paths from a common, and limited, precursor, and ROBINSON (1936) suggests that this precursor forms one C_6 unit of the final C_6—C_3—C_6 structure, the remaining C_3—C_6 portion being characteristic of the final substance. Added support for this view is found in the observation that in *Lathyrus* genes have been recognized which result in some cases in a decrease in the amount of anthocyanin and a corresponding increase in the amount of anthoxanthin, and in others in a decrease in the amount of anthoxanthin along with a corresponding increase in anthocyanin (BUXTON, 1932; SCOTT-MONCRIEFF, 1932). However, *Lathyrus* also contains genes which act as specific suppressors of anthoxanthin without affecting the amount of anthocyanin (BEALE, *et al.*, 1939).

An exhaustive criticism of either hypothesis is scarcely justifiable at the present time for two reasons. First, the evidence as to genetically controlled anthoxanthin variations is very scanty, and much of it has been expressed in terms of such generalizations as "anthoxanthins," "copigments" and "ivory" and "yellow flavone," terms which express nothing as to the detailed structure of the substance so designated. Secondly, certain assumptions have occasionally been made concerning the structure of the anthoxanthin present in one color-variation on the basis of a detailed study of another color-variation in the same species (LAWRENCE and SCOTT-MONCRIEFF, 1935).

That such assumptions may lead to error is evident from the observation made in the present work that the flavonol pigment present in some white varieties of the carnation and that present in a bright yellow variety ("Maine Sunshine") are identical. This substance, kampferol (V) (3,5,7,4′-tetrahydroxyflavone), which occurs in the flowers as a glycoside of undetermined nature, is evidently responsible for little or none of the actual pigmentation of the yellow flower. The pigment of the yellow flower is apparently not flavonoid in nature and its character is still unknown. Approximate methods of identification (for example the "ammonia test"),[5] coupled with the bright yellow color of the flowers may have led to the designation of the pigment of this flower as the "yellow flavone" and similar methods applied to the white varieties may have resulted in the conclusion that these contained an "ivory flavone." In both acyanic and anthocyanin-colored variations careful examination is necessary to determine the nature of the flavonoid substances present.

In the cases of the studies on *Dahlia* it is significant to note that what was called in the earlier work the "yellow anthoxanthin" is probably the chalcone, butein, which was later isolated from a yellow form (PRICE, 1939). The provisional and partial structure which had been assumed for the "yellow anthoxanthin" contained the phloroglucinol residue common to most of the anthocyanidins and to the "ivory flavone" which had been isolated earlier; butein, however, contains the resorcinol nucleus, a striking, and certainly important, change from the standpoint of geneticochemical relationships.

[5] Although polyhydroxy flavones and flavonols are not deeply colored their salts are intensely yellow. Consequently, when a flavone-containing white flower petal is exposed to ammonia fumes it becomes yellow; a yellow petal deepens in color.

The point to be emphasized is that very little exact information is available concerning the combined genetic and chemical relationships of pigments other than those of the anthocyanin type. The reasons for this are obvious. The very nature of the anthocyanins lends them to genetical studies since color variations for which they are largely responsible can be easily followed either by inspection of the flowers in the field or with the aid of the simple and accurate tests devised by ROBINSON for the analysis of anthocyanin types on flower-petal extracts. No such ready means for determining either the amount or the nature of the anthoxanthins is yet available. Genetically different white flowers are often difficult or impossible to distinguish in the field and the simple tests available for determining the presence or absence of pigments of the anthoxanthin type can give only an approximate idea of the amount of pigment present and are totally inadequate for determining with any degree of certainty the structure of the compound. If mixtures are present information obtained by simple tests is likely to be more misleading than useful.

Aside from the obvious advantages to be derived from the acquisition of exact information regarding the anthoxanthins present along with the anthocyanins in a colored flower, it seems clear for reasons given earlier that in a species which includes white and yellow (acyanic) color types as well as anthocyanin-colored varieties, the acyanic types deserve equal consideration if an attempt is to be made to correlate chemical structure and pigment concentration with genetical data.

In the course of the investigation of the inheritance of flower color in the carnation (*Dianthus caryophyllus*, Linn.) a genetical analysis of the acyanic (white and yellow) types has been made (MEHLQUIST and GEISSMAN, 1947). Some of these are available for chemical study, and more will be available as their genotypes are established and they can be produced in sufficient quantity.

These genetical studies on color-variation in the carnation have been summarized (MEHLQUIST, 1939) and have recently been described in more detail (MEHLQUIST and GEISSMAN, 1947). The colors fall into three main groups: (1) the acyanic group, composed of the whites and yellows; (2) the transition group in which the color is due to a small amount of pale anthocyanin on yellow background; and (3) the cyanic group in which full anthocyanin colors from salmon to magenta occur. In addition to these there are found variegations which include flaking, striping, shading and the production of various picotee patterns. With one exception (possibly two) the genes which govern the development of these variegations appear to be multiple-allelic to the six main genes governing color variation. These genes have been represented by *Y*, *I*, *A*, *S*, *R* and *M*. So far as the basic genes are concerned the genotypes of the three color groups may be represented thus:

Acyanic group: *Y I a; y I A; y I a; Y i a; y i A; y i a*
Transition group: *Y i A*
Cyanic group: *Y I A*

In the latter two groups the gene pairs *S-s*, *R-r*, and *M-m* bring about modifications in the shade or intensity of the color. Their effects on the members of

the acyanic group are not obvious by inspection of the flowers and it is hoped that chemical studies will reveal any effects which may exist.

The present report is an account of a broad preliminary survey covering determination of (A) the identity of the anthocyanidins and (B) of the anthocyanins of the cyanic flowers (including several variegated forms), (C) some experiments on the application of spectrophotometric and colorimetric methods to the determination of relative and absolute pigment concentrations in crude petal extracts of cyanic forms, (D) an examination of a genetically-controlled variation in pigment concentration in a single color type, and (E) the determination of the identity of the flavones in several selected color forms.

EXPERIMENTAL METHODS AND RESULTS

Most of the flowers used in this study were grown for this purpose in the Ornamental Horticulture experimental gardens of the UNIVERSITY OF CALIFORNIA, Los Angeles. In a few instances flowers of standard commercial varieties obtained in commercial establishments were used. Only such varieties were used whose genotypes were either already known from earlier genetical work or are in the process of being established.

Immediately after collecting the flowers the petals were cut off at the calyx so as to remove the lower, chlorophyll-containing portion of the petal. The discarded portion generally contained little or no anthocyanin. In some cases the fresh petals were used; in others they were dried (a) in air until brittle and then for a short time at 45°C, or (b) in a vacuum desiccator over sulfuric acid. Post-mature and field-dried flowers were not used.

A. Cyanic Varieties—Establishment of the Nature of the Anthocyanidins.

The determination of the anthocyanidin types in the cyanic forms was carried out by the use of the methods developed by ROBINSON and ROBINSON (1931, 1932, 1933, 1934). The fresh petals were ground with sand and extracted with dilute (1 percent) hydrochloric acid. The filtered extract was boiled for about one minute with an equal volume of concentrated hydrochloric acid and the cooled solution extracted first with ether, then with amyl alcohol. The amyl alcohol extract was washed with 1 percent hydrochloric acid, diluted with benzene and the pigment was extracted by washing with 1 percent hydrochloric acid until the benzene layer was nearly colorless. The process was repeated and the final aqueous solution of the anthocyanidin was thoroughly washed with benzene.

The color tests carried out included (a) the color change observed on shaking some of the solution with amyl alcohol, followed by the addition of sodium acetate, (b) the color produced with ferric chloride, (c) extraction with the "cyanidin reagent," and (d) the "oxidation test."

A typical experiment is as follows: To a 1 percent hydrochloric acid extract of *Crimson* petals was added an equal volume of concentrated hydrochloric acid and the solution boiled for about one minute. The cooled solution was washed thoroughly with ether and the anthocyanidin transferred to amyl alcohol. The amyl alcohol layer was separated, diluted with much benzene and

shaken with 1 percent hydrochloric acid. The pigment was again transferred from the aqueous solution to amyl alcohol and again transferred to 1 percent hydrochloric acid. The final aqueous solution was washed thoroughly with benzene. The following tests were performed on samples of the aqueous solution:

1. The solution was shaken with amyl alcohol in the presence of added sodium acetate. The color of the organic layer was red-violet. A drop of ferric chloride solution was added, the mixture shaken and the layers allowed to separate. The amyl alcohol layer was a pure, deep blue.

2. The solution was shaken with "cyanidin reagent" (a mixture of toluene (4 volumes) and cyclohexanol (1 volume)). The organic layer acquired a rose-red color but color still remained in the aqueous layer.

3. A sample of the aqueous solution was shaken with air, half its volume of sodium hydroxide solution (10 percent) was added, followed at once by concentrated hydrochloric acid and amyl alcohol. The pigment was recovered and appeared in the amyl alcohol layer upon shaking the mixture.

These results are characteristic of *cyanidin.*

Only two anthocyanidins have been reported to occur in the carnation color forms previously examined by others (ROBINSON and ROBINSON, 1931; BEALE, *et al.*, 1941) and only two were found in the color forms examined in this work. These are pelargonidin and cyanidin, the first of which is found in the "salmon," "red," "light pink," and "deep pink" forms; and the second in the "lavender," "crimson" and "magenta" forms.

The following color forms were examined (Table 1.):

TABLE 1

COLOR	GENOTYPE	ANTHOCYANIDIN
Salmon	*Y I A s r m*	Pelargonidin
Red	*Y I A S r m*	Pelargonidin
Light pink	*Y I A s r M*	Pelargonidin
Deep pink	*Y I A S r M*	Pelargonidin
White variegated red (random)	*Y I* a^{var} *S r m*	Pelargonidin
White variegated red (picotee)	*Y I* a^{var} *S r m Pic*	Pelargonidin
Lavender	*Y I A s R m*	Cyanidin
Crimson	*Y I A S R m*	Cyanidin
Lavender	*Y I A s R M*	Cyanidin
Magenta	*Y I A S R M*	Cyanidin
White variegated crimson (random)	*Y I* a^{var} *S R m*	Cyanidin
White variegated crimson (picotee)	*Y I* a^{var} *S R m Pic*	Cyanidin

It can be said with a considerable degree of certainty that in flowers containing pelargonidin no cyanidin occurs. The highly characteristic blue color produced by the action of ferric chloride on cyanidin solution makes the detection of small amounts of this pigment easy. The pelargonidin-containing extracts studied showed none of this characteristic blue color when treated with ferric chloride. That the cyanidin-containing flowers contain no pelargonidin cannot be inferred by similar observations, since pelargonidin produces no

characteristic color with ferric chloride and small amounts of it in a cyanidin solution would produce no striking modification of the cyanidin reaction. Evidence from another source indicates, however, that in the cyanidin-pigmented magenta (*VIASRM*) carnation the cyanidin (as the glucoside, cyanin) is unaccompanied by pelargonidin derivatives. This conclusion is based upon the observation (see Experimental Part, C.) that spectrophotometric comparison of an unpurified petal extract of magenta carnations with a solution of crystalline cyanin chloride showed that the absorptions of these solutions were practically identical over the range 470–530 mμ.

The ether solutions obtained by the extraction of the hydrolyzed petal extracts, as described above, were evaporated to dryness and the residues taken up in alcohol and treated separately with ferric chloride and with sodium hydroxide solution. Yellow colors with alkali and colors with ferric chloride ranging from brown-green to olive-green were obtained in every case, indicating the presence of substances of the flavone type.

B. Cyanic Varieties—Establishment of the Nature of the Anthocyanins.

ROBINSON and ROBINSON (1931) and BEALE, PRICE and STURGESS (1941) have examined a number of carnation color forms, working with named commercial clones (varieties) of genotypes unknown to or unspecified by them, and have reported the presence in various clones[6] of four anthocyanin types: pelargonidin-3-monoside and -3,5-dimonoside, and cyanidin-3-monoside and -3,5-dimonoside. These observations have been confirmed on known genotypes of the same colors as those tested by ROBINSON and have been extended to some forms not previously examined.

The tests were carried out on freshly collected petals. Samples of these were ground with sand and allowed to stand with 0.5–1 percent hydrochloric acid. The suspensions were all filtered through Super-cel; subsequent treatment of the filtrate varied from case to case. Usually a thorough washing of the aqueous extract with ethyl acetate was the first step, followed in the case of the diglycosides with an amyl alcohol extraction, to remove flavonoid substances. The monoglycosides were purified by removing them from the aqueous extract with a 5 percent solution of picric acid in cyclohexanone and returning them to 1 percent hydrochloric acid after dilution of the cyclohexanone-picric acid solution with petroleum ether. The final dilute hydrochloric acid solutions of the pigments were in every case washed thoroughly with benzene and then with ether, followed by removal of residual dissolved ether under reduced pressure.

The tests employed were those described by ROBINSON and ROBINSON (1931) and by ROSENHEIM (1920), and included (a) observation of the distribution of the pigment between the aqueous solution and amyl and butyl alcohols, with and without the addition of sodium chloride, (b) observation of the color changes brought about by the addition of sodium acetate, sodium carbonate,

[6] The clones (varieties) used by ROBINSON were "Mary Allwood" (salmon); "Brilliant," "Aviator" and "Spectrum" (red); "Improved Ward" (deep pink); "Nigger" and "Topsy" (crimson). Those used by BEALE, *et al.*, were not specified by them.

sodium hydroxide, and ferric chloride solutions to portions of the aqueous extract. In the cases of some of the monoglucosides the test for complex (acylated) sugar residues was performed.

A typical experiment is as follows: A sample of 5.75 g. of the dried, ground petals of the *Scarlet* form was allowed to stand overnight with 30 ml. of 0.5 percent hydrochloric acid. The centrifuged solution was filtered through Super-cel and washed thoroughly with ethyl acetate and with ether. The anthocyanin was extracted with a 5 percent solution of picric acid in cyclohexanone and returned to 1 percent hydrochloric acid after dilution of the cyclohexanone solution with petroleum ether. The resulting aqueous solution was then washed thoroughly with benzene and ether (to remove picric acid) and the last traces of ether removed under diminished pressure.

The final solution was scarlet in color. The pigment was only *slightly* extracted by an equal volume of amyl alcohol but to a considerable extent (not completely) by butyl alcohol. After the addition of salt the pigment was *completely* extracted by butyl alcohol.

The addition of sodium acetate to the solution changed the color to a violet-red, changing to a deeper brownish-red with sodium hydroxide. Ferric chloride did not markedly change the color of the original extract.

The pigment was stable to short treatment at the boiling-point of the solution with sodium hydroxide.

These observations, coupled with identification of the anthocyanidin as pelargonidin in a separate experiment, show that the Scarlet form contains a *pelargonidin-3-monoside.*

The results obtained are as follows (Table 2):

TABLE 2

COLOR	GENOTYPE	ANTHOCYANIDIN	SUGAR-TYPE
Salmon	*Y I A s r m*	Pelargonidin	3-monoside
Red	*Y I A S r m*	Pelargonidin	3-monoside
Light pink	*Y I A s r M*	Pelargonidin	3, 5-dimonoside
Deep pink	*Y I A S r M*	Pelargonidin	3, 5-dimonoside
Lavender	*Y I A s R m*	Cyanidin	3-monoside
Crimson	*Y I A S R m*	Cyanidin	3-monoside
Lavender	*Y I A s R M*	Cyanidin	3, 5-dimonoside
Magenta	*Y I A S R M*	Cyanidin	3, 5-dimonoside
White variegated crimson (random)	*Y I* a^{var} *S R m*	Cyanidin	3-monoside
White variegated crimson (picotee)	*Y I* a^{var} *S R m Pic*	Cyanidin	3-monoside

The nature of the tests carried out does not permit the identification of the sugars present in the anthocyanins. Further, positive proof that such somewhat unusual glycosides as rhamnosides or pentosides were present would have necessitated exact measurements of the distribution coefficients of the pigments between amyl alcohol and water. In view of the findings of ROBINSON

(1931), BEALE (1941) and others it is felt that such refinements were not necessary and that the work of these investigators could be used as the justification for accepting qualitative evidence which agreed with their results.

C. Spectrophotometric and Colorimetric Determination of Pigment Concentrations.

With a view to ultimately extending measurements of pigment concentration to all of the cyanic forms of known genotype, experiments have been performed to determine the relative accuracies of spectrophotometric and colorimetric measurements using crude petal extracts and comparing them with standard solutions of the pure, crystalline anthocyanin the flowers used were known to contain. The flowers chosen were magenta (*YIASRM:* cyanidin dimonoside) and lavender (*YIAsRM:* cyanidin dimonoside). The standard was a sample of crystalline cyanin chloride, isolated from deep-red rose petals and purified by the method of ROBINSON and TODD (1932). The instruments employed were a Beckman Photoelectric Spectrophotometer and a Fisher Photoelectric Colorimeter equipped with a green filter.

A determination of the absorption spectrum of cyanin chloride was carried out by measuring the transmissivities of solutions prepared by diluting with 1 percent hydrochloric acid, a standard stock solution of the crystalline pigment ($C_{27}H_{31}O_{16}Cl \cdot 3H_2O$) in 1 percent hydrochloric acid. Measurements were made over the range 400–540 mμ, using a cell 1.30 ± 0.01 cm in cross-section. The values of ϵ (molar extinction coefficient) were calculated from the expression $\epsilon = \dfrac{\log I_0/I}{cl}$, where c = moles/liter, l = 1.30 cm. Values of I_0/I below 8.0 were found to deviate significantly from the averages obtained in a series of varying concentrations and were dropped in determining ϵ values. The best average values are tabulated below (Table 3):

TABLE 3

λ(mμ)	$\epsilon \times 10^{-3}$
400	4.40
470	14.30
480	18.75
490	24.20
500	28.45
510	31.15
520	30.60
530	26.00
540	17.50

Magenta. (*YIASRM*) (A) A 1.00 g. sample of the dried petals was ground to a fine powder and extracted with cold 1 percent hydrochloric acid until the petal meal was colorless. The extract was clarified by centrifuging and by filtering it through a layer of Super-cel, and finally made up to 500 ml in a

volumetric flask. The absorptions of four solutions prepared by diluting 0.50, 1.00, 1.50, and 2.00 ml of this stock solution to 10 ml with 1 percent hydrochloric acid were measured in the spectrophotometer. Using the values of ϵ found for cyanin chloride, values for concentration were calculated for each of the four solutions using measurements made at 10 mμ intervals over the range 470–530 mμ. The values found were as follows (Table 4):

TABLE 4
Magenta.

ml stock solution	0.5	1.0	1.5	2.0
ml 1% HCL	9.5	9.0	8.5	8.0
λ(mμ)	concentrations found: (moles/liter)$\times 10^6$			
470	3.47	7.38	10.36	13.83
480	3.53	7.31	10.34	13.64
490	3.40	7.07	9.91	13.06
500	3.39	6.87	9.89	13.13
510	3.36	6.80	9.80	13.18
520	3.41	6.89	9.98	13.50
530	3.44	7.03	10.22	13.51
Average:	3.43	7.05	10.07	13.41
Conc. of stock solution (calc.): (moles/1×10^6)	6.86	7.05	6.72	6.71

Average: 6.84×10^{-6} mole/1

From these values the pigment concentration in the dry petals is calculated to be 2.40×10^{-2} gm/gm dry petals.

(B) Colorimetric measurements were made on the same magenta stock solution, using as a standard the cyanin chloride solution. Four solutions having concentrations of 1.00, 0.50, 0.25, and 0.125 (stock 1.00) gave the following values in gm pigment/gm dry petals: 2.52, 2.46, 2.44, 2.32 ($\times 10^{-2}$); average 2.43×10^{-2}. These values are in sufficiently close agreement to show that the relatively rapid and convenient colorimetric measurement is capable of an accuracy adequate for the purpose of exact comparisons between different color forms.

Of considerable significance is the close agreement between the individual concentrations calculated for each wave-length in the spectrophotometric measurement. Since these values are calculated from values determined on pure cyanin chloride it is evident that the shape of the absorption curve for the crude petal extract must be almost exactly that of the curve for the pure pigment. The presence of appreciable amounts of pelargonidin glycosides would cause a definite shift in the curve toward shorter wave-lengths. The small deviations which do occur may be due to slight co-pigment effects due to substances other than anthocyanins present in the crude extracts.

Lavender. (*YIAsRM*) (A) Spectrophotometric measurements were made on a crude petal extract (clarified only) prepared from 0.764 gm of dry petals and made up to 100 ml with 1 percent hydrochloric acid. The following figures were obtained (Table 5.):

TABLE 5
Lavender.

ml stock solution	5	5	5	10
ml 1% HCl	15	10	5	5
λ(mμ)	concentration $\times 10^6$ (moles/l)			
490	3.21	4.05	5.96	7.35
500	3.08	4.03	6.00	7.40
510	3.11	4.05	6.02	7.45
515	3.16	4.12	6.11	7.68
520	3.18	4.21	6.29	7.95
530	3.39	4.53	6.84	8.85

From these figures the concentration of the stock solution is calculated to be 12.55×10^{-6} moles/l and the pigment content of the petals 0.115×10^{-2} gm/gm dry petals.

(B) Colorimetric determination of the concentration of the above stock solution gave a value from which the pigment content of the petals was calculated to be 0.130×10^{-2} gm/gm dry petals. A considerable variation (from 0.152 to 0.124 ($\times 10^{-2}$)) between the values found for various dilutions of the stock solution indicated a considerable deviation of the readings from the standard curve (concentration vs. colorimeter reading) drawn from measurements taken on the pure pigment. A second stock solution prepared from 1.00 gm of dry petals, collected at another time, gave the same average value, 0.130×10^{-2} gm/gm dry petals, and the same variation (0.158 to 0.112 ($\times 10^{-2}$)) over the range of dilutions used.

These variations are interesting in that they show what is probably a co-pigment effect. In the spectrophotometric measurements the absorption markedly increases in the longer wave-lengths (cf. 530 mμ, Table 5), an effect reflected in the "bluing" effect of co-pigments. That a pronounced effect was observed in the colorimetric measurements is probably due to the fact that the green filter used selected a spectral region in which the deviation of the absorption curve of the crude petal extract from that of the pure pigment was becoming marked.

D. Intensity in a Single Color-Type.

There have been segregated in the course of genetic studies three red genotypes differing in shade and identified as *salmon-red*, *standard-red*, and *deep-red*. These are all of the general genotype *YIASrm* and may vary either (a) in having multiple-allelic "*S*" genes, or (b) an inhibition gene (*N*). Qualitative tests have shown that these are all pigmented by pelargonidin and there

is reason to believe that all of them contain this anthocyanidin in 3-monosidic combination.

The pigment concentrations in the *salmon-* and *standard-reds* were determined relative to that in the *deep-red* (= 1). Aqueous extracts (1 percent HCl) of weighed amounts of the dried petals were made up to known volumes. Using a Fisher Photoelectric Colorimeter, a standardization curve was drawn using as a standard the *deep-red* solution prepared by making up the extract of 1.00 g of the dried petals to 250 ml with 1 percent HCl. Comparisons of the readings made with solutions of the other two reds made up in the same way gave the following results:

DEEP RED (1.00 G MADE UP TO 250 ML)

SOLUTION	READING	CONCENTRATION
Stock	87.4	1.00
*	74.7	0.50
*	56.2	0.25
*	36.7	0.125
*	21.3	0.0625
*	11.3	0.0312

STANDARD RED (1.00 G MADE UP TO 250 ML)

SOLUTION	READING	CONCENTRATION
Stock	76.6	
*	57.3	
*	37.5	0.526 (ave.)
*	22.4	
*	12.4	
Stock†	76.3	
*	56.2	
*	35.8	0.500 (ave.)
*	20.3	

SALMON-RED (1.00 G MADE UP TO 250 ML)

SOLUTION	READING	CONCENTRATION
Stock	57.5	
*	36.7	0.242 (ave.)
*	20.8	
*	10.5	

* Each solution was prepared by diluting the preceding one to twice its volume with 1 percent HCl.

† Different sample, gathered at another time from different plants.

These results indicate that the pigment concentrations are in the ratio 1:2:4 within the limits of fairly good agreement, in this series of variations. A probable error of up to about 5 percent is indicated in the agreement to that extent between the two samples of standard red, gathered and made up in solution at different times.

E. The Flavone Constituents of Several Selected Color-Forms.

(*A*) *White.* The white flowers used were a mixture of one or more *yIar*, *YIAr* and *YIar* genotypes whose *S-s* and *M-m* constitutions were unknown. The petals were cut off just above the calyx, dried and ground. Extraction of 84 gms (393 gms fresh) with petroleum ether and then with ether resulted in the removal of waxy constituents but these extracts contained no substance soluble in alkali with a yellow color. This shows that the flavone derivatives present in the flowers are all in glycosidic combination.

Extraction of the ether-extracted meal with 95 percent alcohol yielded a brown solution which was evaporated under reduced pressure. The syrupy residue was taken in water and treated with lead acetate. No precipitate was formed, indicating that flavone derivatives having the 3′, 4′-dihydroxy grouping were probably absent. After removal of the lead with hydrogen sulfide the solution was concentrated to 100 ml and refluxed for two hours, with the addition of 1 ml of concentrated sulfuric acid. The brown precipitate was dissolved in alcohol, ether was added and the alcohol was removed by washing with water. Evaporation of the ether layer yielded an oily residue which after solution in alcohol and precipitation with water was obtained as a somewhat amorphous yellow solid weighing 0.55 gm. It gave a yellow solution in alkali, a green-brown color with ferric chloride, and a red color when reduced in alcoholic solution with magnesium and hydrochloric acid. When a solution of this substance in alcohol was treated with aqueous lead acetate some precipitate was obtained. Not precipitated, and recovered from the alcoholic solution, was 0.22 gm, isolated as a yellow, crystalline material, m.p. 245–255°. A part of this substance was sublimed at 200–220°/2mm, yielding two fractions, one melting (with decomposition) at 275–276° (kampferol) and the other at 259–260°. Acetylation of the lower-melting fraction yielded kampferol tetraacetate, m.p. 182–183.5°.

Anal. Calc. for $C_{23}H_{18}O_{10}$: C, 60.78; H, 4.00. Found, C, 61.08, 60.97; H, 4.25, 4.30.

The acetate did not depress the melting point (182–184°) of an authentic sample of kampferol tetraacetate. Acetylation of the remainder of the crude material yielded kampferol acetate, m.p. 182–183°.

The lead precipitate was decomposed with acetic acid and from it were isolated two substances. One of these was crude kampferol which was converted into its acetate, m.p. 182–183.5°. The other was a white, acidic substance, m.p. 354° (dec.). It gave a violet-red color with acetic anhydride-sulfuric acid and a pink color in standing with concentrated sulfuric acid. It appears to be a sapogenin acid.

Anal. Found, C, 72.30, 72.53; H, 9.30, 9.45; $C_{27}H_{42}O_5$ requires C, 72.58; H, 9.50.

These results indicate that kampferol is the only flavone substance present in the white forms tested. No evidence for the presence of any other flavone or flavonol was obtained.

(*B*) *Yellow.* The petals from 500 "Maine Sunshine" (yellow; probable geno-

type $\frac{Y}{y}\ \frac{i^{var}}{i}\ \frac{a^{var}}{a}\ \frac{s}{s}\ \frac{r}{r}\ \frac{M}{M}$) flowers were separated and dried, yielding 250 g. of dry material. A 100 g sample of this was extracted successively with petroleum ether, ether, methanol, ethanol and dilute ethanol (50 percent), and the last three extracts were combined and evaporated to a syrup. The residue was dissolved in 125 ml of water and saturated lead acetate was added until no further precipitate formed. The small amount of dirty grayish-tan precipitate was removed and the filtrate treated with basic lead acetate. The bright yellow precipitate was collected and made up to a suspension in 100 ml of water, 20 ml of 6 N sulfuric acid was added and the mixture was heated on the steam cone for several hours. The lead sulfate was collected and extracted with alcohol and the aqueous solution was extracted with ether. The combined alcohol and ether extracts were washed thoroughly with water and the resulting (alcohol-free) ether solution washed with aqueous sodium bicarbonate, dried and evaporated. A yellow solid was obtained. This was divided into two approximately equal portions. One of these weighed 60 mg and yielded 130 mg of colorless acetate, m.p., after two recrystallizations from alcohol, 183.5–185° (kampferol acetate). The other portion (70 mg) was recrystallized twice from dilute alcohol, forming pale yellow needles, m.p. 272–275° (dec.); it gave the characteristic color-reactions of kampferol with magnesium-hydrochloric acid (scarlet), concentrated sulfuric acid (yellow, strong green fluorescence) and ferric chloride (olive-green).

The precipitate obtained with neutral lead acetate may have contained a trace of quercetin (the magnesium-hydrochloric acid color had a somewhat more bluish tone than that given by kampferol), but none of this could be isolated.

These results are striking proof that the structure of the flavone constituents of acyanic flowers cannot be deduced from the flower color. The term "ivory flavone" and "yellow flavone" are meaningless unless supported by chemical identification of the substances in question. There is now no reason to suppose that one might not find white flowers containing quercetin (or luteolin) and yellow flowers containing kampferol (or apigenin). Possibly the genes *S-s*, *R-r*, and *M-m* are critical in determining the constitution of the flavone constituents in both white and yellow forms. The genotypes necessary for an examination of this possibility have not yet been available but are in process of preparation for future studies.

(*C*) *Crimson.* The commercial variety "Woburn"

$$\left(\text{probable genotype } \frac{Y}{y^{fl}}\ \frac{I}{i}\ \frac{A^{var}}{a}\ \frac{S}{S}\ \frac{R}{r}\ \frac{m}{m}\right)$$

was used. A 100 gm sample of the dried petals was extracted successively with ether, ethyl acetate (which removed only traces of flavones) and methyl alcohol. The alcoholic extract was treated portionwise with lead acetate and then basic lead acetate, discarding the first fractions (violet to blue) which contained the bulk of the anthocyanin. The last two fractions were worked up

separately. The last fraction yielded only dark, brown, tarry materials after hydrolysis and no crystalline substance could be isolated from these.

The middle fraction was hydrolyzed with dilute acid and the liberated flavones taken up in ether. Fractional precipitation of this material with neutral and basic lead acetate yielded two main fractions:

1. Neutral lead acetate. Hydrolysis of this precipitate, isolation of the flavone and acetylation yielded about 100 mg of a crystalline acetate, m.p. 183.5–189°. This was separated by fractional crystallization into five fractions, m.p. 189–193°, 192–193.5°, 192–193.5°, 191–193.5°, and 187–197°. Recrystallization of the first four fractions yielded a product, m.p. 193–194.5°, which was quercetin pentaacetate (mixed m.p. unchanged.).

2. Basic lead acetate. Hydrolysis of this precipitate and acetylation of the ether-soluble material yielded about 10–15 mg of kampferol acetate, m.p. and mixed m.p. 182.5–184° (mixed m.p. with quercetin pentaacetate, from 163°–).

The significance of the presence in this crimson form of quercetin (in much greater amount than kampferol) and the absence of demonstrable amounts of quercetin in the white and yellow forms examined may lie in the presence of the dominant *R* in the crimson form and its absence in the others. It will be recalled that the presence of *R* in the cyanic forms is associated with pigmentation by cyanidin derivatives, while cyanic forms homozygous for *r* contain pelargonidin derivatives. It is possible, therefore, that the factor *R* may be associated with an oxidation of a precursor at an earlier stage than that at wich the final elaboration of the anthocyanin or flavone structure takes place. It will be of considerable importance to determine the structure of the flavones in yellow and white forms carrying the factor *R*.

DISCUSSION

The experiments described in this paper have shown that the flower petals of the carnation may contain the flavonols quercetin and kampferol, as glycosides, and the anthocyanidins cyanidin and pelargonidin (table 1), both as mono- and diglycosides (table 2). No evidence has been obtained that mixtures of cyanidin and pelargonidin derivatives occur in a single flower and in one case (magenta) definite evidence has been obtained that the cyanidin (as cyanin) is not accompanied by any other anthocyanidin derivative. Mixtures of the two flavonols occur in some cyanic and possibly in acyanic forms, but in the few cases studied one of them has predominated, usually to such an extent that only an indication (e.g., by a color-reaction) of the presence of the other is obtained. In every case examined, the cyanic flowers contain flavones. No "albino" flowers—free of both flavone and anthocyanidin derivatives—have occurred among the color forms so far examined. None of the flowers examined appears to contain carotenoid pigments. A yellow form ("Maine Sunshine") contains, along with kampferol, a yellow pigment which is not flavonoid but the nature of which is still undetermined.

The presence of cyanidin is associated with the presence of the gene *R*; when the recessive *r* is present the anthocyanin is derived from pelargonidin (table 1). These genes have the same effect in variegated forms as in full-colored forms.

The genes *M*(*m*) control the degree of glycosidation of the anthocyanidin, the structure of which is determined by *R*(*r*). Flowers having *M* are (3,5-) dimonosides, while flowers with *m* only are monoglycosides (table 2). Again, these conditions obtain in both full-colored and variegated forms. The known "blueing" effect of the *M* factor depends upon this chemical action of the factor, since it has been recognized by many workers that diglycosides produce distinctly bluer tones in flower petals than do monoglycosides of the same anthocyanins.

Evidence from quantitative studies on magenta (*YIASRM*) and lavender (*YIAsRM*) forms, as well as from inspection of flowers in the field, indicates that the *S*(*s*) genes control the amount of anthocyanin, the constitution of which is determined only by the *R*(*r*) and *M*(*m*) genes. The ratio of the pigment concentrations in the magenta (*S*) and lavender (*ss*) forms is about 20:1 (Experimental Part, C). Further data are needed to show whether this same ratio is always associated with this genetical difference, and whether a different ratio is associated with a heterozygous form (*Ss*) compared with an *ss* form.

No information is at hand at the present time concerning the influence of the *S*(*s*) and *M*(*m*) genes on the nature or amount of flavone present in flowers of the acyanic group. Concerning the effect of the *R*(*r*) genes, some information has been obtained (Experimental Part, E). White flowers of several genotypes but all carrying only the recessive *r*, and one bright yellow flower carrying *r* only, contained the same flavonol, kampferol (V). Aside from the bearing this observation has upon the function of *R*(*r*), this result is important in demonstrating that visual differences in acyanic flowers are not necessarily associated with differences in the flavone derivatives they contain. Until acyanic flowers containing the dominant *R* are available for study no more can be said about the influence of this gene in flowers of this type. In view of the additional observation that a crimson form ("Woburn," having *Rr*) contained quercetin (III), along with a much smaller amount of kampferol, it would appear that the *R*(*r*) gene determines the state of oxidation of the flavone derivative in the same way that it determines the structure of the anthocyanidin. It may be significant that "Woburn" is heterozygous for *R* and contained both quercetin and kampferol. On the basis of this very scanty evidence it might be predicted that a flower having the genotype *RR* would contain quercetin only and a scarlet form (*rr*) would contain kampferol only. These predictions must await further experiments for confirmation.

Since the white flowers used contained a mixture of genotypes among which were both *Y* and *y* forms, it can be said that, since only kampferol was found in these, the *Y*(*y*) genes do not directly influence the structure of the 2-phenyl nucleus of the flavone.

It appears that none of the six basic gene pairs so far referred to is solely responsible for the formation of the fundamental C_6–C_3–C_6 skeleton from which the flavones and anthocyanins may be considered to be derived. This conclusion is based largely upon numerous applications of the "ammonia test" to flowers in the field and in the laboratory, in the course of which forms recessive for at least one of all of the basic factors have come under scrutiny. No

acyanic flower has yet been found which does not show qualitative indications of the presence of flavone derivatives.

The results obtained in the present study resemble in general many of those reported by other workers in studies in other flowers. The data at hand, coupled with information obtained from the fundamental genetic studies (MEHLQUIST and GEISSMAN, 1947) may be summarized as follows:

(1) The complementary genes *Y*, *I* and *A* are necessary for full anthocyanin production. Flowers recessive for *Y* and/or *A* are white. Flowers recessive for *I* and carrying the dominant *Y* and *A* genes are lightly anthocyanin-pigmented on a yellow ground and belong to what is called the "transition group." Flowers recessive for *I* and *A* are pure sulfur yellow. Flowers recessive for *I* and *Y* are pale yellow as are the triple recessives *y i a*.

(2) Flowers carrying *Y*, *I* and *A* produce full concentration of anthocyanin color only in the presence of *S*; flowers carrying the recessive allele *s* are pale in color and include the light pink, salmon and lavender shades. The gene *S*, together with one, possibly two, modifying factors, operating as suppressors, appears to determine the exact amount of anthocyanin produced.

(3) The production of cyanidin derivatives through the gene *R* is dominant to the production of pelargonidin derivatives (*r*).

(4) Flowers having the *M* gene produce anthocyanidin diglycosides, while with the recessive allele, *m*, monoglycosides are formed. This gene operates in the same way whether the anthocyanidin is cyanidin or pelargonidin.

(5) Certain flowers carry one or possibly two inhibition or suppression genes which appear to operate in a strictly quantitative manner. Three different red forms, genetically identical except for the dosage of these suppression genes, contained amounts of anthocyanin which were in the ratio 1:2:4.

The points just discussed are recapitulated in table 6.

INTERPRETATION OF RESULTS

The following comments are based upon all of the information so far available concerning inheritance in the carnation; this includes that from the chemical evidence described in the present paper and from genetical data described in two other papers (MEHLQUIST, 1939; MEHLQUIST and GEISSMAN, 1947).

In considering a possible scheme to account in terms of biosynthetical relationships for the chemical effects produced by the various individual color genes, it will be of importance to determine so far as possible what specific role each gene plays in the elaboration of the final structures of the pigments present in a flower.

The function of the genes *S* (together with one of more modifying genes) appears to be solely one of control of the amount of anthocyanin, whereas the nature of the anthocyanin, both in its state of hydroxylation and the number of sugar residues present, is determined only by *R* and *M* and is independent of the allelic state of *S*. Whether *S* controls the concentration of flavone as well is not yet known.

The gene *M* appears from present evidence to be concerned solely with the change from an anthocyanidin monoglycoside to an anthocyanidin diglycoside.

TABLE 6

FLOWER CARRYING*	PLUS GENE	RESULTING COLOR
YA	*I*	Full anthocyanin color on white (ivory) ground
	i	Pale anthocyanin on yellow ground
YI	*A*	Full anthocyanin color
	a	White
IA	*Y*	Full anthocyanin color
	y	White
iA	*Y*	Pale anthocyanin on yellow ground
	y	Pale yellow
Ya	*I*	White
	i	Yellow
yA	*I*	White
	i	Pale yellow
yi	*A*	Pale yellow
	a	Pale yellow
ya	*I*	White
	i	Pale yellow
ia	*Y*	Yellow
	y	Pale yellow
YIA	*S*	Pelargonidin or cyanidin
	s	Pale pelargonidin or pale cyanidin
YIAS	*R*	Cyanidin
	r	Pelargonidin
YIAs	*R*	Pale cyanidin
	r	Pale pelargonidin
YIA (S or s)	*M*	3, 5-Dimonoside
	m	3-Monoside

* Results have been the same whether the plants tested have been homozygous or heterozyous for the dominant genes.

There are two stages in the anthocyanin synthesis at which this gene may operate: (1) it may effect the union of a sugar residue with a preformed anthocyanidin monoglycoside, or (2) it may direct the earlier stages of the synthesis in such a way that the precursor of the final pigment is a polysaccharide which contains the preformed glycosidic linkages, which then remain unchanged throughout the subsequent synthetic processes and appear in the final product.

It may be possible to choose between these alternatives by an examination of the glycosidic linkages existing in the flavones present in two acyanic flowers which differ only in the *M* gene.

Flowers recessive for *Y* produce no anthocyanin except under very favorable conditions (MEHLQUIST and GEISSMAN, 1947). An intermediate allele y^{vl} produces a flush of anthocyanin when it is accompanied by both *I* and *A*, but in the presence of *y* the flowers are pale yellow or white depending upon whether *I* is present in the recessive or dominant condition.

Since flowers recessive for *Y* do produce flavone derivatives, *Y* is not necessary for the formation of the fundamental C_{15} unit from which the final products are formed. It is conceivable that what is at present regarded as the recessive allele of *Y* is really an intermediate in a series of multiple alleles, of which the as yet undiscovered lowest recessive may lack the power of producing the fundamental precursor; at present, however, there is no evidence to support such an assumption. *Y* cannot be responsible for the degree of oxidation (hydroxylation) of the flavonol since flowers dominant for *I* (and carrying *r*) contain kampferol whether *Y* or *y* is present. In the yellow flowers (i.e., recessive for *I*) the intensity of the yellow color is determined by the allelic state of *Y;* that is, *Y*-yellows are deeper in color than *y*-yellows. The meaning of this observation in terms of the chemical constitution of the flower petal is still not clear, since experiments have shown that the bright yellow color of the flowers is due, in part at least, to an ether-soluble, probably non-carotenoid, non-flavonoid pigment of unknown nature. It is probable that the difference in color between pale yellow and a deep yellow form is not due to a higher concentration of flavonol in the latter, since the flavonol present in the deep yellow "Maine Sunshine" is the same as that in certain white forms.

Flowers recessive for *I* still produce anthocyanin in small amounts, but only when both *Y* and *A* are present in the dominant condition. These cases form the faintly-colored transition group. Not enough cases have been examined in sufficient detail to determine whether the allelic state of *I* determines the degree of hydroxylation of the flavones present, but it appears that this is not the case. Evidence based upon visual inspection of various genotypes indicates that *I* does not affect the composition of the anthocyanins; only *S*, *R* and *M* are concerned with that. In short, the chief difference between two flowers differing genetically only in the allelic condition of *I* seems to be that the color of the flower which is recessive for *I* is modified in the direction of yellow, the other pigments being chemically the same in the two cases. The production of the yellow pigment evidently takes place at the expense of anthocyanin: the transition group (*YiA*) are lightly anthocyanin pigmented on a yellow ground, while the cyanic group (*YIA*) show full anthocyanin development on what may be considered a colorless or white (ivory) ground. The gene *I* thus acts as an inhibitor of the process in which some precursor is elaborated into the yellow pigment; when *I* is present in the dominant condition, then, more of the hypothetical precursor is available for the synthesis of anthocyanin, this route being controlled by other genes.

Flowers recessive for *A* like those recessive for *Y*, fail to produce antho-

cyanin. An allele, a^{var}, allows the production of small amounts of anthocyanin in various variegation patterns, but only when *A* is present in the dominant condition can full anthocyanin be produced. The anthocyanin formed when a^{var} is present is chemically the same as that produced in the presence of *A*, if *S*, *R* and *M* are the same in both cases. It is significant to note that the spots or stripes produced in the variegated forms are full-colored areas.

The effect of *A* upon the nature or concentration of the flavones has not been determined. Its function seems to be only that of control of the total amount of anthocyanin. It may act in some synthetic step in which the particular oxidation-level of the C_3 portion of the fundamental carbon skeleton is determined, and is thus a modifying factor operating in some specific oxidation or reduction step upon a supply of precursor already present as the result of the action of other factors.

The gene *S* is necessary for full anthocyanin formation, flowers recessive for *S* containing the same anthocyanin (other factors being the same) as flowers dominant for *S*, but in lower concentration (approximately one-twentieth as much in (*VIAsRM*) as in (*VIASRM*)). Since the flower of the genotype (*ViAs*) is salmon-yellow and that containing (*ViAS*) is orange, it appears that *S* is not concerned in the production of total pigment but, given a sufficient supply of starting material, acts to modify it along a route leading specifically to anthocyanin.

The function of the *R* gene appears to be one of control of the degree of hydroxylation of the 2-phenyl group. Cyanic flowers dominant for *R* always contain cyanidin derivatives; that is true also in the case of flowers carrying variegation factors which produce anthocyanin-pigmented areas upon white backgrounds. Evidence from a limited number of cases indicates that the nature of *R* affects the degree of hydroxylation in the 2-phenyl group of the flavones as well. This may mean that *R* operates at an early stage in the synthetic sequence through which the flavones and anthocyanins are formed, and directs the degree of hydroxylation of some common precursor.

Many questions remain to be answered before all of the suggestions made in the foregoing discussion can be substantiated or elaborated upon. The answers to many of them will undoubtedly be found in a more detailed examination of certain acyanic forms and comparisons of these with suitably constituted cyanic forms.

SUMMARY

1. Chemical studies have shown that the anthocyanidins of cyanic varieties of the carnation are cyanidin (in *R* forms) and pelargonidin (in *rr* forms).

2. The anthocyanins present in cyanic forms are monoglycosides (*mm* forms) and diglycosides (*M* forms).

3. The anthocyanins in the pigmented areas of variegated forms have been found to be controlled by the *R-r* and *M-m* genotype of the flowers in exactly the same way as in the full-colored forms.

4. The flavones present in carnations are kampferol and quercetin, which are probably present in the petals as glycosides. Acyanic forms having *rr* contain kampferol. No acyanic forms having *R* have been examined, but a crimson

form (having *Rr*) was found to contain mostly quercetin along with a small amount of kampferol.

5. All forms studied have contained some flavonoid constituents; no true albinos have been observed.

6. Three scarlet forms, differing in the dosage of an inhibition gene, were found to contain amounts of anthocyanin (pelargonidin monoglycoside) in the ratio 1:2:4.

7. Some suggestions have been made concerning the possible roles of the color genes in the biosynthetic processes by which the pigments are formed.

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THE EIGHTH INTERNATIONAL CONGRESS OF GENETICS will be held in Stockholm in the summer of 1948. The date will be announced later, when more is known about other congresses planned for 1948. Swedish geneticists have formed an Organization Committee for the Congress, with Professor Gunnar Dahlberg, of the University of Uppsala, as chairman, and Professor Gert Bonnier, Institutet för Husdjursförädling, Wiad, Eldtomta, as general secretary.

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